HISTOLOGY OF THE FEMALE REPRODUCTIVE TRACT OF THE CHEETAH

(*Acinonyx jubatus*)

by

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in the

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Declaration

I, Miles John Penfold do hereby declare that the research presented in this dissertation, was conceived and executed by myself, and apart from the normal guidance from my supervisor, I have received no assistance.

Neither the substance, nor any part of this dissertation has been submitted in the past, nor is to be submitted for a degree at this University nor any other University.

This dissertation is presented in partial fulfilment of the requirements for the MSc degree in the Department of Companion Animal Clinical Studies.

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To my lovely wife Julia for her love and support. And to God the Father, Jesus and the Holy Spirit for making all this possible.

Quote:

Proverbs 22:29 “Do you see any truly competent workers? They will serve kings rather than working for ordinary people.”

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Abstract

Cheetahs are listed as vulnerable by the IUCN (International Union for Conservation of Nature) with their total worldwide population estimated at 6674 individuals. Ongoing habitat destruction, fragmentation, human animal conflict and predation threaten the survival of the wild cheetah population. Zoos and wildlife parks are tasked with maintaining a genetically healthy population as insurance against catastrophic extinctions. In contrast to reproduction in the wild, reproductive success in captivity has been poor, with only a handful of facilities achieving reasonable success. Much research has been done to try identify why. Both husbandry and spermatic factors have been investigated. Recently the focus has shifted to investigating the role of uterine health in reproductive success. Since the identification of pathology in any organ or system is made with reference to the normal, it was decided to investigate and document the normal histology of the female cheetah reproductive tract. Six captive female cheetah uteri, obtained from post-mortems after elective euthanasia of cheetahs aged 7-10.5 years, and one uterus, from a 3 year-old cheetah that died acutely, were sectioned and processed by routine histological methods. Histological sections were stained with haematoxylin and eosin and studied and photographed with an Olympus BX63 light microscope using bright field illumination and fitted with an Olympus DP72 digital camera. Very early signs of cystic endometrial hyperplasia were identified in 4 of the 7 uteri. These sections were disregarded and only sections with no pathological changes were included in the histological atlas. The histology of the female reproductive tract of the cheetah generally resembled that of the domestic dog, cat and African lioness. In the cheetah the suspensory ligament mostly branched in the region of the tubal pole of the ovary with the branch running in the mesosalpinx to join the tip of the uterine horn. The reproductive ligaments were comprised predominantly of smooth muscle, which was very well developed and supported the entire length of the uterus up to the cervix, similar to the African lioness. Cyst-like structures were found in the region of the UTJ. The uterine tube opened into the uterine lumen from a microscopic, well-defined papilla. The papillary glands were morphologically distinct to the uterine glands. The cyst like structures appeared to be dilations of the papillary glands, potentially acting as a storage cistern. The cyst-like structures are of importance to pathologists examining uterine sections in the UTJ region as they could easily be confused with cystic endometrial hyperplasia. It is therefore advised that sections for histopathology of the uterus of the cheetah be taken at least 5 mm distal to the UTJ to preclude accidental inclusion of these cyst-like structures. The *Tunica muscularis* of the uterine tube varied from the dog in that it was well developed anduniformly thick from the

infundibulum to the ampulla, before increasing in thickness in the isthmus. The uterus and uterine glands were lined by a low cuboidal to cuboidal epithelium which contrasts with the dog and lioness. No ciliated cells were present in the uterus. The cervix displayed very few tertiary folds, no mucigenous nor goblet cells which is similar to the lioness but contrasts to the dog. The cervix contained no elastic fibres. Further research needs to be done to document the histology of the vestibule and clitoris as well as to determine the presence or absence of striated muscle in the distal two thirds of the round ligament as described in the lioness. The function of the papillary glands and significance of their cyst like appearance also needs to be further investigated.

Ethics

A Section 20 Permit (no. 12/11/1/1/18) to perform research was obtained from the Department of Agriculture, Forestry and Fisheries. The project was approved by the National Zoological Gardens of South Africa's Research and Ethics Committee (Project no. P13/11) and the University of Pretoria Research and Ethics Committee (Project no. V089-17). A research/collecting permit (1846/2013) was obtained from the Namibian Ministry of Environment and Tourism and the samples were imported into South Africa with the required CITES export (no.0042838) and import (no. 137670) permits, as well as a veterinary import permit (no. 13/1/1/30/2/10/6-2013/11/002397). Once in South Africa, the samples were transported and stored with the required national Threatened or Protected Species (TOPS) ordinary permit (no. 05238).

List of Abbreviations

AI	Artificial insemination
ART	Artificial reproductive technologies
CITES	The Convention on International Trade in Endangered species of Wild Fauna and Flora
cm	centimetre
IUCN	International Union for Conservation of Nature
UTJ	Uterotubal junction
PAS	Periodic acid-schiff
POC	Paraovarian cyst
T.	Tunica
yr	Year

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1. Introduction

There is an overpopulation of the domestic cat (*Felis catus*) in many countries around the world.[1]. Conversely, the majority of wild felids (twenty-three of thirty-six species) are endangered or threatened with extinction in at least part of their natural range [2]. The IUCN Red List classifies most feline species as threatened, vulnerable or endangered [3]. This is primarily due to ongoing habitat destruction, fragmentation, human animal conflict, poaching and use in traditional medicines. Zoos and wildlife parks are tasked with maintaining a genetically healthy population as insurance against catastrophic extinctions. Unfortunately, most wild felids do not reproduce well in captivity [2]. Therefore, assisted reproduction continues to be investigated. ART researched in the felid family include AI, in vitro fertilisation and embryo transfer [1], in vitro culture of preantral follicles [4], in vitro production of embryos [5], intracytoplasmic sperm injection [6], nuclear transfer [7] and cryopreservation of embryos [8]. Oocytes collected from pre-pubertal felines for embryo production can shorten the generation interval, thereby accelerating the rate of genetic advance achievable by either natural selection or the introduction of new genes by transgenesis [9]. Uchikura et. al. work supports the possibility of using ovaries obtained from felines who have died before puberty for ART [10].

Cheetahs are on the critically endangered list of CITES in Iran (60-100 individuals), North, West and Northwest Africa. They are listed as vulnerable in the other parts of their current range in Central, Eastern and Southern Africa [3]. Their total world population is estimated at 6674, with Southern African holding the largest subpopulation estimated at 4190 [3]. This is further divided across 10 subpopulations, the largest consisting of 3940 cheetahs which occupy a transboundary area involving northern South Africa, Namibia, Botswana, south-western Zambia and south-western Mozambique [3].

Major causes for concern include the fragmentation of the cheetah population, the fact that 76% of their territory consists of unprotected land and their low density which ranges from $1/100\text{km}^2$ to $1/4000\text{km}^2$ [3]. This has the potential to end up with subpopulations becoming so small and /or fragmented that they cannot maintain genetic biodiversity. Additionally infant mortality in the wild is extremely high, estimated at as much as 70% due to disease, maternal neglect and predation by other predators [11]. In the Serengeti less than 5% of wild born cheetah cubs reach independence with the vast majority of mortalities as result of predation by lions (*Panthera leo)* [12]. In Namibia, where the highest concentration of the world's remaining wild cheetahs reside (approximately 2500 individuals), ongoing conflict with farmers is likely to determine the long-term survivability of the population in the country [13].

The cheetah already suffers from high genetic uniformity with 10-100 times less variation than other mammalian species. This is most likely as result of a relatively recent population bottleneck and subsequent inbreeding [11,14]. This genetic uniformity has both advantages and disadvantages. Advantages are that deleterious recessive traits may already have been expressed allowing breeding from any combination of individuals to not adversely affect genetic outcome [11]. Allografts have been shown to be 100% compatible between unrelated individuals [11] which is surgically advantageous and convenient should the need arise. Disadvantages include high species vulnerability, not only to expression of deleterious recessive traits, but also a limited ability to adapt to changes in the environment such as the documented susceptibility with high mortality (60% of the population) to a feline coronavirus in Oregon, United States of America [11]. To decrease the risk of extinction, improving breeding efficiency using ART in captivity as well as protecting natural habitat are considered necessary steps [15,16].

Breeding of cheetahs in captivity has proved notoriously difficult [2,11]. Both species specific issues [11] and poor husbandry practices contribute to this poor reproductive performance [12]. The inability to reliably identify when females are in oestrus for timing of breeding introductions is a major problem [2]. Captive bred cheetah cubs suffer from a high infant mortality rate of 29.1% before 6 months. Additionally, normal sperm counts in male cheetahs are 10 times less than in domestic cats and sperm morphology abnormalities average 71% compared to the domestic cats 29% [11].

Cheetah life expectancy varies from around 6 years for wild cheetahs [17] up to 10-15 years for captive raised cheetahs [18]. Cheetahs are non-seasonal breeders with year-round gonadal activity [2] and are induced ovulators that sometimes display spontaneous ovulation [19,20]. They reach sexual maturity at 2–3 years of age and exhibit comparatively short oestrous cycles, which range from 7 to 21 days in duration [2]. Oestrus lasts 2–6 days [2]. Gestation averages 94 days [2] where after 3-4 cubs are usually born [21]. Cheetahs can be paired easily but breeding may inexplicably not occur[1]. Generally breeding females are housed separately today as oestrous cyclicity can be socially suppressed even when aggressive interactions are relatively minor [2]. Nulliparous females older than 7 years are poor candidates for AI, probably as result of uterine aging or diminished oocyte quality [1].

ART successfully used in cheetahs to produce offspring are limited to laparoscopic in utero AI post equine chorionic gonadotropin treatment using fresh and cryopreserved semen which has resulted in a repeatable pregnancy incidence of >45% and is the only ART currently with a high enough efficacy to contribute to genetic management in cheetahs [1,22]. In vitro fertilisation and embryo transfer have rendered inconsistent results in the dependable production of offspring in all felid species and hence is not currently used as a genetic management tool. These low overall success rates (<20%) are mostly attributed to the failure of current hormonal stimulation protocols and highlight the need for ongoing research [1].

The harvesting of preantral follicles in prepubertal cats for maturation in vitro to viable embryo stage has been demonstrated in domestic cats [10]. Extrapolation and development of this technology for use in cheetahs could further accelerate the rate of genetic advancement within the species by decreasing generation interval [9]. The stock piling of ovaries from deceased cheetahs could provide a useful additional genetic material bank for preservation of the species. Retrieval of large numbers of preantral follicles less than 8 hours post mortem via mechanical follicle isolation has been demonstrated in the cheetah [4].

A thorough knowledge of the normal histological structure of the female cheetah reproductive tract is a prerequisite for development and implementation of current and future ARTs.

The use of the domestic cat as a model for wild felids has resulted in significant progress being made in understanding the reproductive biology of wild felids. Detailed assessments of the normal events associated with ovarian anatomy and function in the naturally mated domestic cat have been especially beneficial [1,4].

Much is still unknown about folliculogenesis, such as follicle recruitment signalling and its restriction to a single follicle, the origin of granulosa cells and the mechanism of follicle development in induced versus spontaneous ovulators [23]. Bristol-Gould & Woodruff contend that the domestic cat is an ideal model for the study of folliculogenesis due to the availability of all follicular stages, particularly primordial follicles, at any given point in the domestic cats' lifecycle, as well as the relative ease of procuring ovaries from local veterinary practices following elective ovariohysterectomy. They also contend that further studies on domestic cat ovaries could uncover clues that may aid in elucidating important mechanisms controlling reproduction in endangered felids [23]. Clearly, a thorough knowledge of the normal histological structure of felid ovaries is a prerequisite for ongoing reproductive research in our quest to preserve endangered felids. Additional benefits may include future development of additional reproductive technologies, aimed at curbing ovarian activity, for use in the management of the domestic cat overpopulation problem. The histology of the domestic cat ovary has been described, but in limited scattered sources, primarily in older texts [24]. Additionally, the literature is conflicting and incomplete. Specifically, there is no single reference that definitively summarises this literature in one location. There is also no felid specific follicle classification system. This makes using the domestic cat as a histological reference for comparative studies problematic. This is compounded by the fact that anatomical and histological information is often companioned with sources devoted primarily to dog anatomy and histology [25], leading to the incorrect assumption that the two species are alike.

Jewgenow and Stolte have pointed out that due to differences in ovarian composition and variation in follicle classification systems it is difficult to compare results in felids with those from other species [4]. This further highlights the need to comprehensively document the histology of the ovaries of felids, and for a felid specific follicle classification system, as extrapolation from other species cannot be relied upon.

The suspensory apparatus of the domestic cat has not been as fully histologically evaluated as has that of the African lioness [26]. A notable difference between the two felids is that the African lioness round ligament contains striated muscle in addition to smooth muscle [26], while the domestic cat round ligament contains only smooth muscle [27]. The function and importance of this striated muscle in lionesses remains to be elucidated. It is unknown whether other wild felids such as the cheetah have striated muscle associated with the round ligament.

The purpose of this study is to provide a histological atlas of the ovary, ligaments and tubular parts of the female reproductive tract of the cheetah, which will serve as a useful reference for researchers and pathologists to refer to in the future and may aid in the conservation of the species.

2. Justification

2.1. Literature review

Very few specific anatomical or histological papers on the cheetah have been published. These include papers examining the functional anatomy of the hind limb [28] and forelimb [28] and anatomy of the stifle, shoulder joint [29], brain [30], carpus [31,32], thoracic limb [33] and skull [34]. Histological papers are confined to the striated muscles [35] and sperm [36].

No or limited literature exists on the anatomy and histology of the reproductive organs of the cheetah. Sperm morphology has been documented [36]. Limited histopathological studies include uterine pathology [18,37] and miscellaneous post-mortem samples [38–40].

Anatomy and histology of domestic cat, dog and lioness reproductive structures is more abundant and is used as a reference for investigating the histology of the female reproductive tract of the cheetah.

2.1.1. General anatomical features of the female reproductive tract

The gross anatomy of the female reproductive system of the domestic cat consists of the ovaries attached to the mesovarium and free border of the suspensory ligament, uterine tube running in the mesosalpinx [41] from near the opening of the ovarian bursa to the tip of the uterine horn, uterus comprising two horns that join to form an unpaired body[42] that terminates at the cervix, vagina, vestibule (urogenital sinus) and vulva [27] . Size and location of the ovaries, the mesovarium, oviduct, mesosalpinx, proper ligament of the ovary, mesometrium, round ligament, suspensory ligament, right and left horns as well as body and location of the uterus, cervix, location of the vagina, the vestibule, urethral tubercle, vestibular glands and their openings, fossa of the clitoris, clitoris, vulva and labia as well as the arteries and veins to the female reproductive organs have been described in the domestic cat [27]. The gravid uterus as well as placenta together with its blood supply have also been described [43].

2.1.2. The Ligaments

The suspensory apparatus suspends the uterus from the abdominal wall and consists of the suspensory ligament, proper ligament, round ligament and broad ligament. In the domestic cat the suspensory ligament originates in the middle of the last one or two ribs and extends to the tubal extremity of the ovary where it continues in the mesovarium to join the proper ligament; which connects the ovary to the tip of the uterus; where it continues as the round ligament, which courses caudally in an outpouching of the lateral wall of the mesometrium until it enters the inguinal canal, where it continues to finally terminate in the connective tissue near the vulva [27]. Watson recently noted that, in contrast to the dog, there is no vaginal ring and no vaginal process in the domestic cat. The round ligament was found to terminate in the fascia enveloping the cranial surface of the external pudendal vein cranial to the external pudendal artery within the first 1–1.5 cm of its extra-abdominal course [61]. It is unknown exactly where it terminates in the cheetah as no data has been published in this regard. It terminates on the medial femoral fascia in lionesses [26]. The broad ligament is composed of a double fold of peritoneum that forms a curtain that extends to and surrounds the suspensory, proper and round ligaments as well as the ovary and uterus. It is widest at the ovaries. Nerves, blood vessels and lymphatics run in it to supply the associated reproductive structures. It does not play a supporting role nor suspend the reproductive structures in the abdomen, but rather unites its components [41].

The round ligament contains smooth muscle in the female domestic cat which allows for stretching during pregnancy [27]. Detailed reference to the composition of the suspensory, proper and broad ligaments in the domestic cat could not be found suffice to say they contained smooth muscle [27]. In the dog the suspensory and proper ligaments contain smooth muscle and connective tissue [41]. The broad ligament additionally contains blood vessels, lymphatics and nerves and is covered by peritoneal mesothelium. In the lioness, the round ligament consists of distinct, separate bundles of smooth and striated muscle [26]. The suspensory ligament contains predominantly smooth muscle and loose connective tissue [26]. The histological composition of the proper ligament and broad ligament was not described in the lioness suffice to say the proper ligament contained smooth muscle [26].

The round ligament shows marked hypertrophy during pregnancy in humans and it has been proposed that it should be called the “round muscle”. It plays an active role in maintaining the position of the uterus in humans [62]. This hypertrophy has not been documented in felids.

Striated muscle was found in the caudal portion of the lioness’s round ligament leading that author to postulate that the lioness may be able to voluntarily suspend the uterus closer to the lumbar spine during hunting thereby providing a protective function to any fetuses. The attachment of the round ligament in the lioness to the medial femoral fascia might also explain the behaviour of lionesses post coitus where they roll on their backs and stretch out their legs possibly facilitating the transport of sperm towards the ovaries [26].

The mating behaviour of the domestic cat, cheetah and lioness display similarities. Female cheetahs are receptive to multiple males and allow repeated mating over a few days while oestrus lasts. The male mounts the female and grasps her by the scruff. The female moves into a receptive position with her hind legs positioned under her rump which elevates her hind end. Her tail is moved laterally. The male intromits and thrusts rapidly 20-30 times. The event ends with ejaculation, the male dismounting and the female displaying aggressive behaviour towards the male by rolling onto her back and swatting him on the side of the head. She then rolls from side to side, while remaining on her back, before standing up [63–67].

It remains to be investigated whether striated muscle exists in the round ligament or other parts of the suspensory apparatus of the cheetah and what the exact role the composition of the round ligament or its point of attachment play in the cheetah.

2.1.3. The Ovary

The feline ovary consists of the following histological layers and structures.

Major layers:

- Germinal epithelium
- *Tunica albuginea* (ovarian capsule)
- Cortex (zona parenchymatosa)
- Medulla (zona vasculosa)[44,45]

The germinal epithelium covers the ovarian surface, except at the hilus, and is continuous with the mesovarium. It starts as a simple cuboidal epithelium (during oogenesis) which becomes squamous with age. The germinal epithelium is supported by the *Tunica albuginea*, a dense, white, fibrous irregular connective tissue that may have a lamellar orientation. Beneath this the ovarian cortex begins and extends to the medulla [44,45].

The ovarian / cortical stroma consists of or contains the following structures:

- Loose collagenous tissue resembling areolar connective tissue.

- Spindle-shaped fibroblasts arranged in whorls, orderly arranged around follicles or vessels or orientated parallel to the ovarian surface. These fibroblasts are pleomorphic and adaptive and can assume epithelioid characteristics as seen by their formation of the follicular theca and interstitial gland cells.
- Oocytes – consisting of a nucleus and ooplasm – and derived by mitotic division from oogonia primarily before but also shortly after birth.
- Follicles at different stages of development.
- Anovular and atretic follicles.
- *Corpora lutea*.
- Scar tissue from atretic follicles or involuted *Corpora lutea*.
- Interstitial glands in mature animals.
- Blood, nerve and lymphatic supply [25,44,45].

The medulla:

- Is highly vascularised, contains fibroelastic connective tissue, reticular and elastic fibres, connective tissue cells and smooth muscle.
- *Rete ovarii* may be present, are derived from the mesonephric tubules, and consist of channels lined by cuboidal epithelium or short, solid cords of epithelial cells [44,46–48]. They are most often found within the hilus but may also be found within the mesovarium. Their function is speculative [49].
- Hilus cells – groups of epithelioid cells found close to the *Rete ovarii* in the region of the hilus located at the mesovarial attachment are present in some mammals and possibly secrete androgens [47,50].
- Non-myelinated nerves from the sympathetic system originating from the renal and aortic plexuses innervate blood vessels, follicles, *Tunica albuginea* and *Corpora lutea*[46]. The relationship of the parasympathetic system to the ovary is poorly understood but ganglion cells have been observed in the medulla. Although the majority of the nerve supply is vasomotor, some sensory fibres have been observed.
- Blood supply is via the ovarian artery which enters at the hilus. Branches extend to the cortico-medullary junction where they form an extensive plexus. Branches extend from this plexus into the cortex to supply the theca of developing follicles, *Corpora lutea* and stromal elements. Capillary networks completely surround developing follicles and form an extensive network inside *Corpora lutea*. Venous drainage mirrors the arterial setup but veins may coalesce to form a venous plexus in the medulla before exiting the hilus.

- Lymphatics are intimately associated with the theca externa of developing follicles and exit the hilus having passed radially through the medulla. They drain through the lumbar lymph nodes [44,45].

2.1.4. Folliculogenesis

Oogenesis is the process by which primary oocytes are produced by mitotic division of oogonia, mostly before birth but also for a short period after birth in cats, and are embedded in clusters [46,49] in the cortical stroma just beneath the *Tunica albuginea* [47,48,50]. During development of the ovary primordial germ cells migrate from the yolk sac of the endoderm of the embryo to the germinal ridges where they undergo mitosis to form several generations of identical cells known as oogonia. Oogonia enter the first meiotic division but are arrested in prophase and are now known as primary oocytes which consist of a nucleus and ooplasm [44,45]. The majority of primary oocytes regress at this stage leaving no scar tissue [45,50], while the others grow and become surrounded by a single layer of flattened mesodermal cells called follicular cells and become as a general rule quiescent until puberty [25,]. The primary oocytes are now known as primordial follicles [44,45].

With the onset of puberty and its associated hormonal changes cohorts of primordial follicles start to undergo waves of development and regression throughout the reproductive life of the domestic cat. Only a small minority of these follicles will ultimately undergo ovulation. The remainder will regress through a process of atresia [25,46]. The process of selection of follicles for further development has yet to be elucidated [46].

Folliculogenesis describes the process of changes associated with growth and development of primordial follicles as follows. Primordial follicles consisting of a primary oocyte surrounded by a single layer of follicular cells that start to grow under the influence of follicle stimulating hormone [50]. As soon as the follicular cells become mitotically active they become known as granulosa cells [44]. The granulosa cells multiply and differentiate to form different histological structures and layers thereby enlarging the developing follicle whose stromal elements form the follicular theca. Some of these thecal cells, known as the thecal cone, become orientated towards the ovarian surface and displace any superficial follicles as the developing follicle enlarges. The granulosa cells immediately surrounding the ovum become columnar in nature and form the *Corona radiata* with its associated thick, PAS-positive, amorphous basement membrane called the *Zona pellucida* [44]. Cytoplasmic extensions from the *Corona radiata* traverse this basement membrane to interact with the oocyte and are thought to provide nutritional support [49]. The other granulosa cell layers progress from squamous to cuboidal to stratified forming the membrana granulosum surrounded by a basement membrane which separates it from the *Theca interna* (an extensive vascular network surrounding large, epithelioid cells) and *Theca externa* (a layer of fibroblasts) jointly known as the theca folliculi, which forms by stromal cell differentiation [25,44,45]. The stratified *Stratum granulosum* starts to develop Call-Exner bodies, intercellular vacuoles filled with what is thought to be the precursor to *Liquor folliculi*. It accumulates *Liquor folliculi* extracellularly in clefts which ultimately coalesce to form one large cavity, the antrum, which surrounds the ovum [44]. The follicular theca continues to differentiate, its fibroblasts changing into androgen producing epithelioid cells in the *Theca interna* and remaining as a dense coat of fibroblasts in the *Theca externa*. The granulosa cells are divided by the developing antrum into a parietal layer which does not directly surround the developing ovum and differentiates into polyhedral shaped cells and forms the *Stratum granulosum*. The *Stratum granulosum* has the ability to convert androgens formed by the *Theca interna* into oestrogens. The ovum is located to one side of the fluid filled antrum embedded in a mound of granulosa cells known as the *Cumulus oophorus* or *Discus proligerus* which constitutes the visceral portion of the granulosa cells [44–46]. At this point the follicle has passed through the secondary and tertiary (vesicular) stage of folliculogenesis and is a mature or Graafian follicle. It has grown down into the depths of the cortex displacing cortical elements out of its way as it expands.

Multi-oocyte follicles (a Graafian follicle with two oocytes present) have been reported as being frequently observed in cats [10]. Graafian follicles have also been reported in new born kittens [51], 23-day old kittens [52] and one kitten < 20 days old [10]. As the Graafian follicle matures the granulosa cells stop proliferating, having formed a distinct stalk attaching the *Discus proligerus* to the ovum, but the production of *Liquor folliculi* continues [24]. This results in an increase in pressure in the antrum in which the ovum surrounded by the *Corona radiata* floats freely [50] having detached from the stalk of the discus proligerus. Thinning of the *Tunica albuginea* and thecal components occurs and is thought to be mediated by luteinizing hormone induced collagenase, protease and plasmins which allows the follicle to bulge from the ovarian surface. The follicle, now known as a pre-ovulatory follicle, is visible to the naked eye, and the ovum would have completed its first meiotic division with expulsion of its associated polar body resulting in an ovum containing a secondary oocyte [25,44]. The second meiotic division will only take place with sperm penetration of the *Zona pellucida*. The follicular stigma is the part of the follicle that bulges from the ovarian surface and is where ovulation takes place [44,45].

With continued enzymatic degradation the follicular stigma eventually ruptures extruding the ovum surrounded by the *Zona pellucida* and *Corona radiata* into the coelom surrounding the ovary in a process known as ovulation [24,46]. Acute hyperaemia, contracture of the hilus musculature and enzymatic changes at the point of rupture play a combined role [25]. The pressure in the antrum apparently plays no role in ovulation but the follicular fluid may aid transport of the ovum to the infundibulum with the help of the fimbriae [44,45]. The domestic cat, cheetah and most other felids are induced ovulators and require repeated vaginal stimulation, usually through repeated intromission over a few days to induce ovulation [2,20,46,53].

2.1.5. Corpus luteum

Immediately post ovulation the follicle collapses with extensive folding of the wall and pyknosis of some of the granulosa cells. The antrum fills with blood which clots to form a transient structure called the *Corpus haemorrhagicum* [46]. This blood clot is invaded by stromal cells and blood vessels which resorb it and lay down reticular fibres. The granulosa cells and cells of the *Theca interna* start proliferating, hypertrophying and producing lutein (yellow pigment) and other lipids in a process known as luteinisation and are now known as granulosa lutein and theca lutein cells respectively. The resultant structure is highly vascular and is referred to as the *Corpus luteum.* The smaller *Theca lutein* cells are situated peripherally or dispersed as septum like clusters intimately associated with the larger and predominant *Granulosa lutein* cell population[44,45]. Eventually the two luteal cell types become mixed in the *Corpus luteum* and are difficult to distinguish. Both produce progesterone [46].

The fate of the *Corpus luteum* is dependent on the reproductive success or failure of the animal and is referred to as a *Corpus periodicum* (cyclic corpus luteum) if fertilisation does not occur, or gravid *Corpus luteum* if it does. Eventually both of these *Corpora lutea* regress (regressive *Corpus luteum*) being replaced by connective tissue, leaving a *Corpus albicans* behind [25,44,45,50]. The first signs of this regression occur in late diestrus and are characterised by condensation of lutein pigment, which appears reddish (*Corpus rubrum*), conspicuousness of vascular connective tissue and hypertrophy followed by sclerosis of luteal artery muscle cells to leave behind a connective tissue scar [46,54].

2.1.6. Atresia

Less developed follicles that do not ovulate during this cycle and multi-oocyte follicles undergo atresia. Follicles at any stage of the development cycle can undergo atresia. Follicles in an advanced stage of development that undergo atresia leave a scar called the

Corpus atreticum. Primordial follicles do not leave a scar and simply undergo dissolution [44,45].

Anovular follicles, also known as atretic follicles, have an irregular outline, the granulosa cells separate, *Theca interna* hypertrophies, the *Zona pellucida* swells and folds and the oocyte liquefies [44,45]. Nuclear pyknosis and chromatolysis are prominent signs of follicular wall cell atresia. The basement membrane between the *Theca interna* and granulosa cells thickens, folds and hyalinises to form the glassy membrane [46]. Blood vessels invade the follicle. Phagocytic and fibrotic activity results in resorption of the follicle and formation of the *Corpus atreticum* [44,45].

The continuum of atresia is better described in the lioness [26] and human literature [55].

Very early atretic changes

- Intact *Theca interna* and membrana granulosa.
- Some granulosa cells begin to slough into the antrum.
- Antrum still contains follicular fluid.
- Disruption of the *Cumulus oophorus* occurs and degeneration of the ovum advances.
- Swollen *Zona pellucida* is present.
- Occasionally the remnant ovum, surrounded by a swollen *Zona pellucida* can be seen free in the antrum [55].

Advanced early atresia

- Hypertrophy of the epitheloid cells of the *Theca interna* [55]. Distinct layering within the *Theca interna* is present in lionesses [26].
- Absent membrana granulosa; all of its cells have sloughed off and have been resorbed leaving behind the basement membrane.
- The basement membrane has now thickened, and is called the hypertrophied glassy membrane [55]. This does not occur in the lioness and no distinction can be made between the glassy membrane and its hypertrophied state [26].
- Loose connective tissue grows in from the stroma partially filling the reduced antral cavity.
- Follicular fluid is still present.

With moderate atresia

- Stroma replaces the *Theca interna* cells.
- Hypertrophied glassy membrane becomes thicker [55]. In the lioness it regresses and disappears having only gradually thickened and never having reached a hypertrophied state [26].
- Loose connective tissue with small blood vessels completely fills the former antrum.

Late atresia

- Loose connective tissue is also replaced by stroma.
- The hypertrophied glassy membrane remains for some time as the only indication of the former follicle.
- In humans the glassy membrane folds together with hypertrophy [55].
- In the lioness no scarring from completely atretic and resorbed follicles is present[26].
- In the lioness the glassy membrane regresses completely during moderate atresia [26] and its shape has not been described.

The interstitial glands (interstitial endocrine cells) [49] are derived from the *Theca interna* of atretic antral follicles or from granulosa cells of atretic preantral follicles and are located in the cortex [46]. They consist of cords of interstitial endocrine cells which are small, round to polyhedral, epithelioid cells with a round nucleus that stains poorly [46,48]. They are thought to supply prepuberal animals with oestrogens necessary for the development of secondary sex characteristics. They may also be a source of oestrogen associated with the postovulatory period [44,45]. They have not been noted in the lioness [26], although in that study none of the lionesses had ovulated. It remains to be documented if interstitial glands are present in lionesses that have ovulated.

2.1.7. Folliculogenesis classification systems

Histology textbooks and articles use various terms to try divide the continuum of follicle development into easy to recognise stages. Terms are often used interchangeably and synonyms can be confusing as they do not always refer to the same morphological start and end point. This lack of clear definition and standardisation of terms makes comparison of data between authors challenging as the reader needs to become familiar with the authors definition of each term or use of an unknown term. For example, the squamous cells surrounding the primordial follicle that multiply to form the *Stratum granulosum* are referred to as granulosa cells by Lintern et. al. [56], pregranulosa cells and then granulosa cells when they become cuboidal by Bristol-Gould & Woodruff [23], and start as follicular cells and are then called granulosa cells at the secondary follicle stage by Banks [45].

Classification of folliculogenesis as described in commonly available veterinary histology textbooks

Different populations of follicles are present in the cortical stroma. In broad groups they consist of primordial, primary, secondary, tertiary and mature follicles [44,49].

Primordial follicles [44,45,47–50], also referred to as quiescent [44,45] or unilaminar, preantral, resting follicles [46], are the most numerous and least developed follicles in the ovary requiring hormonal stimulation to develop further. Histological layers include the:

- Primary oocyte (derived from the oogonia).
- Surrounded by a flattened layer of follicular cells – a simple squamous epithelium [44–46].

Growing follicles [24,50] refers to primary and secondary follicles [50].

Primary follicles [24,25,44,45,47,49], also referred to as unilaminar, preantral, growing follicles[46,49]. Histological layers include the:

- Enlarging primary oocyte.
- Cuboidal [44–46] or columnar follicular cells.
- Yolk granules are present in the ooplasm.
- Stromal elements surround the follicle [44,45].

Late primary follicles, also known as multilaminar follicles consists of the primary oocyte, *Zona pellucida* and follicular cells with fluid filled spaces between them [47]. The *Zona pellucida* is mentioned as becoming present by Aughey and Frye sometime during the primary follicle stage [48].

Secondary follicles [44,45,47–49], also referred to as multilaminar, preantral, growing follicles [46], consist of the:

- Primary oocyte.

- *Zona pellucida* [44–46,49].
- *Membrana granulosa* – which produces fluid known as the liquor folliculi [44,45]. Priedkalns & Leiser refer to this layer as granulosa cells [46].
- Differentiation of the stromal elements into the *Theca interna* and *Theca externa*[44,45]. Priedkalns & Leiser contend that this differentiation occurs in the next stage [46].
- Basement membrane between thecal and membrana granulosa cells.
- Thecal cone - an accumulation of thecal cells directed towards the ovarian surface which may displace superficially positioned follicles [44,45]. Priedkalns & Leiser do not mention these structures [46].
- *Corona radiata* [47]. Other texts contend that this layer only becomes present in the tertiary follicle stage [44–46,49].

Tertiary follicles [45,47–49] or vesicular follicles [24,25,45] also known as multilaminar, antral, growing follicles [46] or graafian follicles [46–48] or mature follicles [47,48], consist of:

- Primary oocyte.
- *Zona pellucida*.
- *Corona radiata*.
- *Stratum granulosum* - the term now used to refer to the *Membrana granulosum* – the cells are polyhedral but the basal layer may be columnar [44,45].
- Start and maturation of the antrum [44,45] – Call-exner bodies, present in some of the granulosa cells, are large PAS-positive inclusions which represent the intracellular precursors of *Liquor folliculi* [46]. However the term is used in other texts [44,45] to refer to small lakes or intercellular clefts with *Liquor folliculi* between the granulosa cells which become confluent to ultimately form the antrum. Priedkalns & Leiser contend that the start of the antrum is a characteristic of secondary follicles and that the mature antrum characterises tertiary follicles [46].
- Basement membrane.
- *Theca folliculi* composed of the *Theca externa* and *Theca interna* derived from stromal cells.
- The *Theca interna* – a vascular layer containing spindle-shaped cells in the early tertiary follicles [46].
- *Theca externa* – also derived from the cortical stroma and consists of fibrous connective tissue and concentrically orientated, spindle-shaped stroma cells [25] (fibrocytes) [46]. Banks contends that thecal differentiation is initiated in the secondary follicle stage [44,45].
- *Cumulus oophorus* [45] also known as the *Discus proligerus* [24].

Mature follicles [44–46,49,50], also known as pre-ovulatory follicles [44,45] or Graafian follicles[44,50] or late tertiary follicles [46]. Histological layers include:

- All the above-mentioned constituents.
- The follicle protrudes from the ovarian surface and extends to the depths of the cortex.
- The antrum is large and its wall is attenuated [44,45].
- *Corona radiata* and ovum – now attached to the cumulus by a stalk [50,57] or floating freely in the antrum [49].
- *Theca interna* – many of these cells adjacent to the granulosa cells enlarge into polyhedral epithelioid cells typical of steroid producing cells, having lighter chromatin and more distinct nucleoli than their spindle-shaped precursors and contain lipid droplets. An extensive blood and lymphatic capillary network is present [46].
- *Theca externa* – with fibrocytes concentrically arranged around the *Theca interna* and blood vessels supplying capillaries to the *Theca interna*. [46]

What is immediately apparent is that the morphological changes and classification terminology used to try group these changes provide a broad category classification system. Although informative and useful in giving readers a general overview of morphological changes during folliculogenesis, it does not provide a concise or easy to use system for more detailed differentiation of follicle classes as compared with Lintern et. al. [56]. They also fail to address morphological steps that do not consistently occur at a set stage, such as the appearance of the antrum, which may occur as early as the secondary (class D1 – Lintern et. al. classification system) follicle stage [56].

Furthermore, there has been no clear adoption of any one classification system with researchers adapting proposed classification systems to suit their research needs. These systems are based on criteria such as follicle and oocyte size, cell numbers and layers and morphology of cells. Two commonly adapted classification systems include: Pedersen and Peters proposed classification system in the mouse based on follicle cell numbers and oocyte size [57] (Figure 2.1) and Lintern-Moore et. al. classification system using morphological description in human infants [56] (Figure 2.2).

2.1.8. Pedersen and Peters follicle classification system in the mouse [57]

Small follicles:

Type 1 follicle:

- Small oocyte (diameter < 20 um) with no follicle cells attached to its surface.

Type 2 follicle:

- Small oocyte with a few cells attached to its cell surface, but not a complete ring of cells.

Type 3a follicle:

- A complete ring of follicle cells surrounds the oocyte which is usually small or might have started to grow.
- Not more than 20 follicle cells on the largest cross-section.

Medium-sized follicles:

Type 3b follicle:

- One complete ring of follicle cells surrounds a growing oocyte (diameter 20-70 um).
- 21 to 60 cells on the largest cross-section.

Type 4 follicle:

- Two layers of follicle cells surround a growing oocyte.
- 61 to 100 cells on the largest cross-section.

Type 5a follicle:

- A transitory stage between medium-sized and large follicles. In some of the follicles the oocyte is still less than 70 µm, in others it has reached its full size already.
- This type has three layers of follicle cells.
- 101 to 200 cells on the largest cross-section.

Large follicles:

Type 5b follicle:

- A fully grown oocyte (70um) is surrounded by many layers of follicle cells.
- 201 to 400 cells on the largest cross-section.
- No follicle fluid.

Type 6 follicle:

- A large oocyte with many layers of cells.
- The follicle cells are separated by scattered areas of fluid.

- 401 to 600 cells on largest cross-section.

Type 7 follicle:

- A follicle with a single cavity containing follicle fluid.
- There are more than 600 cells on the largest cross-section.
- *Cumulus oophorus* (but not the stalk) has formed.

Type 8 follicle (pre-ovulatory follicle):

- A large follicle with a single cavity with follicle fluid and a
- Well-formed cumulus stalk [57].

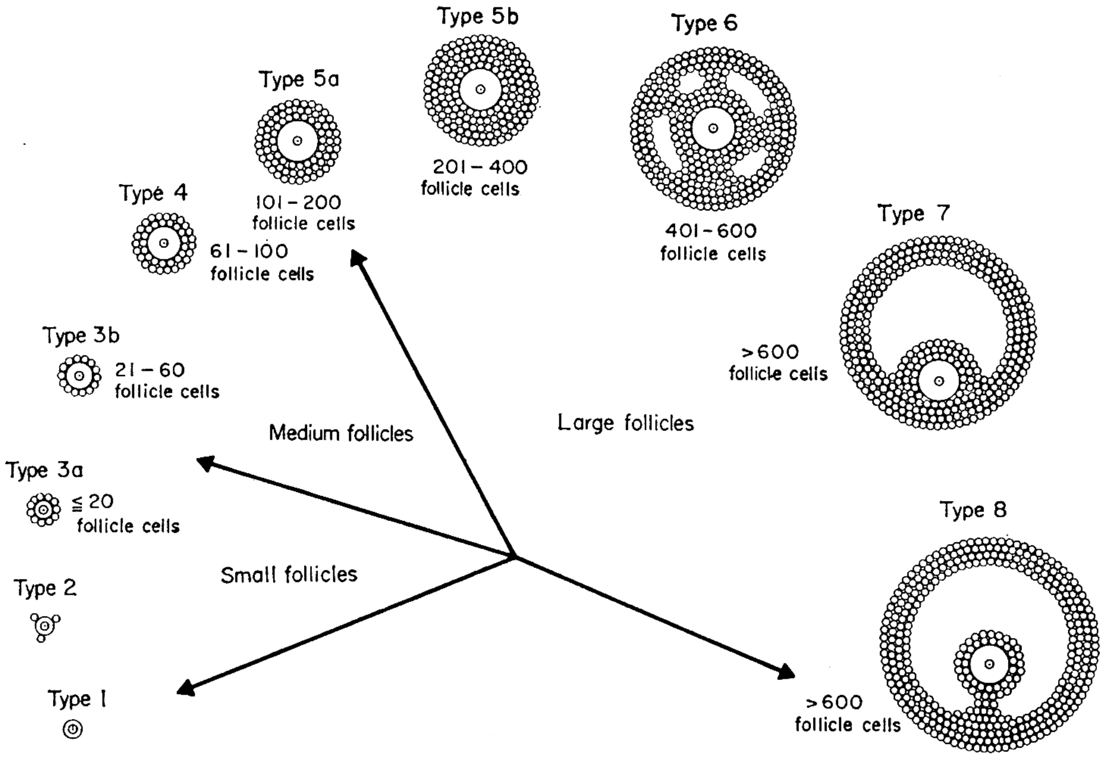


Figure 2.1: Pedersen and Peters proposed follicle classification system in the mouse. The classification is based on (1) the size of the oocyte (2) the size of the follicle defined by the number of cells constituting the follicular envelope, and (3) on the morphology of the follicle [57].

2.1.9. The Lintern-Moore et. al. follicle classification system in human infants (ignoring oocyte and follicle diameters)

Class A follicles:

- Primary oocyte.
- No associated granulosa cells.

Class B follicles:

- Primary oocyte.
- Surrounded by a single layer of flattened granulosa cells.

Class B/C follicles:

- Primary oocyte.
- Surrounded by a single layer of both flattened and cuboidal cells.

Class C follicles:

- Primary oocyte.
- Surrounded by a single layer of cuboidal granulosa cells.
- Separated from the surrounding stroma by a complete basement membrane.
- Surrounding stroma forms a halo of connective tissue fibres and fibroblast-like cells.
- No definitive theca is present.
- Oocyte has not commenced growth.

Class D_1 follicles:

- Primary oocyte commences growth.
- Surrounded by 2-7 layers of granulosa cells.
- *Zona pellucida* is present.

Class D_2 follicles:

- Similar follicle and oocyte size range to D1 follicles but;
- Granulosa cell layer contains fluid filled spaces and Call-Exner vacuoles.

Class E follicles:

- Primary oocyte has ceased growth in most cases.
- Definitive crescent shaped antrum.
- Theca may be well vascularised.
- Follicle size may range from D_1 follicle size up.

Class F follicles:

- Primary oocyte has ceased growth.
- Large fluid-filled antrum.
- Oocyte is embedded in cumulus of granulosa cells.
- Advanced vascularisation of the theca.

- In some cases the thecal cells are markedly hypertrophied [56].

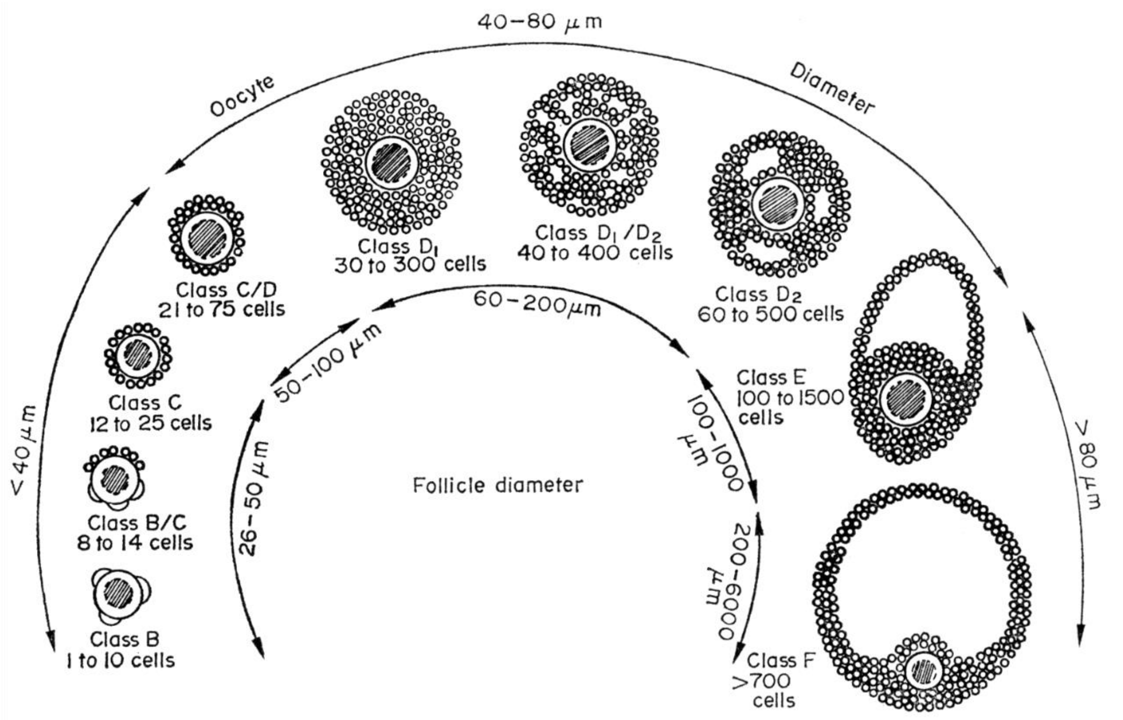


Figure 2.2: Lintern-Moore et. al. follicle classification system in human infancy and childhood. Follicle classification criteria included follicle diameter, oocyte diameter, granulosa cells numbers in the widest cross-section and follicular morphology. Notice that there are no classes for pre-ovulatory follicles as the subjects had not entered puberty. Follicular growth occurred first during the transition from Class C to Class C/D. Oocyte growth commenced during the Class C/D to D transition. Oocyte growth in general ceased during the progression from Class D to Class E but in exceptional cases occurred as early as Class C/D [56].

No follicle classification system has been proposed in the domestic cat [58] nor any wild felids. Bristol-Gould and Woodruff have adapted criteria used in humans and mice for classification of follicles in the domestic cat [58]. Their classification system is based on Lintern-Moore et. al. [56] work and focuses primarily on morphology of the cells. Lintern-Moore et. al. [56] classification system in the investigators opinion reflects functional development of the follicle better than systems relying on follicle or oocyte size or follicle cell numbers alone as these could vary between species.

This investigator proposes the following follicle classification system to describe and compare folliculogenesis in felids. It uses histological criteria as outlined by Lintern-Moore et. al. [56] above. Two additional follicle classes have been added to reflect pre-ovulatory changes in mature animals, one using histological criteria from Pedersen and Peters [57] and the other histological criteria from the literature [49,50,57]. It also incorporates commonly used terms and synonyms to make the text more easily understandable to a wider audience (Table 2.1). Oocyte, follicular size and follicular cell numbers were not used in the criteria.

Table 2.1: Proposed feline specific follicle morphological classification system. * Only morphological criteria from Lintern-Moore et. al. have been used (class A-F). Oocyte and follicle size and commencement of oocyte growth as well as follicle cell numbers have been omitted. The final two thesis terms (blue shading) and their associated suggested class and criteria have been chosen using observation of a distinctive stalk [50,57] and a free floating ovum prior to ovulation noted in the literature [49,50]. ** Differentiation between tertiary and mature follicles is not made by some of the authors. The interchangeability of the terms and their linkage to different morphological criteria outlined previously in the text highlights the need for a more standardised and updated classification system of feline follicles (green shading).

Dissertation terms	**Lintern-Moore et. al. Class** [56]	**Criteria ***	**Commonly used terms & synonyms ****
Primary oocyte	Class A oocyte	Primary oocyte with no associated granulosa cells.	*Primary oocyte; Oogonium.*
Primordial follicle	Class B follicle	Primary oocyte with one layer of squamous granulosa cells.	*Primordial follicle* [44,45,47–50]*; Quiescent follicle* [44,45]*; Unilaminar, preantral, resting follicles* [46]*.*
Early primary follicle	Class B/C follicle	Primary oocyte with one layer of squamous & cuboidal granulosa cells.	
Primary follicle	Class C follicle	Primary oocyte with one layer of cuboidal granulosa cells and a complete basement membrane separating them from a halo of connective tissue fibres and fibroblast-like cells with no definitive theca present.	*Primary follicle* [24,25,44,45,47,49]*, Unilaminar, preantral, growing follicle* [46,49]*.*
Late primary follicle	Class D1 follicle	Oocyte with 2-7 layers of granulosa cells a clearly differentiated thecal layer and a *Zona pellucida*.	*Late primary follicle; Multilaminar follicle* [47]*.*
Secondary follicle	Class D2 follicle	Call-exner vacuoles present in the granulosa cells and or fluid accumulation between the granulosa cells.	*Secondary follicle* [44,45,47–49]*; Multilaminar, preantral, growing follicle* [46]*.*
Early tertiary follicle	Class E follicle	Crescent shaped antrum.	
Tertiary follicle	Class F follicle	Cumulus oophorus is present and thecal cells may be hypertrophied. Advanced vascularisation of the theca is present.	*Tertiary follicle* [45,47–49]*; vesicular follicle* [24,25,45]*; multilaminar, antral, growing follicle* [46]*; Graafian follicle* [46–48]*; Mature follicle* [47,48]*.*
Graafian follicle	Class G follicle	*Cumulus oophorus* has developed a distinctive stalk [50,57].	
Pre-ovulatory follicle	Class H follicle	Ovum is floating free in the antrum. Antral wall bulges from the ovary surface [49,50].	*Mature follicle* [44–46,49,50]*; Pre-ovulatory follicle* [44,45]*; Graafian follicle* [44,50]*; Late tertiary follicle* [46]*.*

Although over 200 papers have been published on gonadal cycle patterns in just over half the felid species [2], few papers have been published on the histology of the reproductive structures. One dissertation on the normal histology of the female lionesses (*Panthera leo)* reproductive tract has been completed [2]. Another paper describes follicle structure up to the preantral secondary follicle stage in domestic cats [59]. The histology and morphometry of folliculogenesis has been described in depth in domestic cats [60]. However, only follicle diameter, follicle stage, oocyte diameter and *Zona pellucida* thickness were used together with Pedersen and Peters follicle classification system for the mouse to determine the main stages of folliculogenesis in the domestic cat. Other histological structures were not mentioned and no multi-oocyte follicles were observed in that study [60]. Many books on the histology of the ovary of domestic animals have been published. The domestic cat is mentioned in these, the assumption being that the ovarian structure unless stated otherwise is similar to that of the dog. However, no single reference contains an in-depth description of all the histological structures purported to be present in the domestic cat. In particular, the stages of atresia have not been described as exhaustively as in humans [55] in the domestic cat but have been described in the lioness [26].

2.1.10.The Tubular parts

Macroscopic components of the tubular parts of the female reproductive tract described in the domestic cat include: the uterine tube (infundibulum, ampulla and isthmus), uterus, cervix vagina, vestibule with major and minor vestibular glands, clitoris with paired *Corpora cavernosa,* and vulva with labia covered by skin rich in apocrine and sebaceous glands. All of the above structures have their own associated glands, nerves, lymphatics and blood vessels [42].

Histologically the uterine tube is comprised of the *Tunica mucosa-submucosa, T. muscularis* and *T. serosa.* The uterus is comprised histologically of the endometrium (functional & basal zone), myometrium and perimetrium (serosa). The cervix and cranial vagina are comprised histologically of the mucosa-submucosa, propria-submucosa, *T. muscularis* and *T. serosa*, whereas in the caudal vagina the *T. serosa* is replaced by the *T. adventitia*. The vestibule possesses similar histological layers to the caudal vagina. The cervix is composed of fibroelastic tissues and venous plexus [42].

2.1.11.The Uterine tube

A comparative anatomical study of the mammalian uterotubal junction among eight species of mammals (pig-tail and rhesus monkeys, pig, dog, rat, rabbit, cattle, sheep) did not include the domestic cat nor any other feline [68].

What follows is a summary from Priedkalns and Leiser Textbook of Histology on the female reproductive system of domestic mammals [42]. The uterine tube or oviduct connects the ovarian bursa to the proximal uterine lumen and conveys the ova, spermatozoa and zygotes and consists of 3 segments:

- A large funnel-shaped infundibulum with finger like projections called fimbriae. These become engorged with blood at the time of ovulation and have the ability to sweep over the surface of the ovary as result of rhythmic smooth muscle contractions. The function of the fimbriae is to trap the ovulated ovum and direct it into the infundibulum. Once inside it is directed down the infundibulum by ciliated epithelial cells which mostly beat towards the uterus until it enters the ampulla.
- Movement through the thin-walled ampulla is primarily facilitated by ciliary activity, although smooth muscle contractions are involved in some species. Fertilisation takes place in the caudal part of the ampulla.
- The fertilised ovum (zygote) enters the narrow, muscular isthmus which joins the uterus. Movement of the zygote to the uterus is effected primarily by muscular contractions, although ciliary activity may be involved in some species. The phase of the oestrous cycle determines directionality of the contractions. Anti-peristaltic contractions during the follicular phase move the luminal content towards the ampulla. Whereas peristaltic contractions during the luteal phase propel the zygote towards the uterus. Uterine and isthmus wall contractions account for the movement of spermatozoa to the ampulla as intrinsic sperm motility plays no role [42,46].

The following layers constitute the histological structure of the domestic mammalian oviduct as summarised from Priedkalns and Leiser Textbook of Histology [42] on the female reproductive system of domestic mammals:

Tunica mucosa-submucosa:

So named because the absence of the thin *Lamina muscularis*, which separates the mucosa from the submucosa in other tubular structures outside of the female reproductive tract, results in the mucosa being continuous with the submucosa. It is highly, longitudinally folded in the ampulla with up to 40 primary, secondary and tertiary folds. These gradually disappear in the isthmus until just 4-8 primary folds are present in the section of the isthmus embedded in the uterine wall. The propria-submucosa consists of loose connective tissue with many mast cells, eosinophils and plasma cells. The mucosal epithelium is simple columnar or pseudostratified columnar epithelium. Microvilli are present on all surface cells, both on the predominant motile ciliated cells and sparser non-ciliated secretory cells. Both cell types occur more commonly in the cranial portion of the uterine tube. During the luteal phase the non-ciliated secretory cells become taller than the ciliated cells. The secretions nourish the ovum and zygote.

Tunica muscularis:

Inner circular smooth muscle layer – occurs throughout the oviduct and gives off radial strands into the mucosa-submucosal layer. The layer is thin in the infundibulum and ampulla but markedly thickens in the isthmus to finally blend with the uterine circular smooth muscle layer.

Outer longitudinal smooth muscle layer – occurs throughout the oviduct, the layer is uniformly thin, strands are isolated and oblique branches occur.

Stratum vasculare:

The *Stratum vasculare* is interposed between the inner and outer smooth muscle layers of the *Tunica muscularis.*

Tunica serosa:

Contains many blood vessels and nerves. The blood vessels form subepithelial vascular complexes which proliferate during pregnancy. Lymphatics form capillary networks in the mucosal and serosal layers which drain into the lumbar lymph nodes. Myelinated and non-myelinated nerves with many submucosal branches are derived primarily from the sympathetic system.

2.1.12.The Uterus

The following section is a summary of Priedkalns and Leiser Textbook of Histology [42] on the female reproductive system of domestic mammals.

The following layers constitute the histological structure of the uterus:

- Endometrium (*Mucosa-submucosa*) – Consists of two zones:
    - Basal zone – A thin, deep layer which generates or restores the functional zone during pregnancy and oestrus.
    - Functional zone –
        - The superficial layer which is generated in response to hormonal changes in the oestrus cycle. Consists of:
            - Simple columnar surface epithelium and simple, coiled, branched tubular glands. These glands are lined by simple columnar ciliated glandular epithelium. These cells comprise both secretory and non-secretory ciliated cells. The branching and coiling is stimulated by oestrogen but copious glandular secretion generally requires progesterone stimulation.
            - Subepithelial, superficial part – richly vascular loose connective tissue rich in fibrocytes, macrophages and mast cells with neutrophils, eosinophils, lymphocytes and mast cells also present.
        - Deep layer – Loose connective tissue that is less cellular than the superficial part.
- Myometrium – Smooth muscle cells are arranged in two layers separated by a the *Stratum vasculare*:
    - Outer longitudinal smooth muscle layer.
    - *Stratum vasculare* – This may be contained in the inner circular layer. This and all preceding layers are continuous with corresponding structures constituting the broad ligament.
    - Inner circular smooth muscle layer.

  The muscle layers hypertrophy and muscle cells increase in number during pregnancy.

- Perimetrium (serosa) – Peritoneal mesothelium covers loose connective tissue which supports multiple blood vessels, nerves and lymphatics.

The myometrium's vascular layer supplies arteries, veins and lymphatics to the endometrium. Numerous nerves, lymph and blood vessels are also present in the perimetrium. The nerves are mainly sympathetic and originate through the uterine and pelvic plexuses and innervate all three layers of the uterus. Parasympathetic supply is from the sacral spinal cord via the pelvic plexus.

2.1.13.The Cervix

The following section is a summary of Priedkalns and Leiser Textbook of Histology [42] on the female reproductive system of domestic mammals.

The cervix is thick walled, muscular and rich in elastic fibres. The mucosa-submucosa forms high primary folds with secondary and tertiary folds. Uterine glands are not present. The cervical glands are mostly mucigenous. The following layers are present:

- Mucosa-submucosa – simple columnar epithelium with many mucigenous cells, including goblet cells. A small portion of the epithelium is ciliated in some species.
- *Propria-submucosa* – dense, irregular connective tissue. This becomes oedematous during oestrus assuming a loose areolar structure.
- *Tunica muscularis* – Continuous with that of the uterine body and vagina.
  - Inner circular smooth muscle layer – has prominent elastic fibres as well.
  - Outer longitudinal smooth muscle layer.
- *Tunica serosa* – mesothelium supported by loose connective tissue.

2.1.14.The Vagina

The following section is a summary of Priedkalns and Leiser Textbook of Histology [42] on the female reproductive system of domestic mammals.

The vagina is a muscular tube with flat longitudinal mucosal-submucosal folds that extend throughout its length. Vaginal mucus originates from the cervix. The following layers are present:

- Mucosa-submucosa - Stratified squamous epithelium that thickens during oestrus and proestrus.

- *Propria-submucosa* - Loose or dense irregular connective tissue containing lymphatic nodules in the cranial vagina.
- *Tunica muscularis*:
    - Thin longitudinal inner layer of smooth muscle.
    - Thick circular layer of smooth muscle separated into bundles by connective tissue.
    - Thin outer longitudinal layer of smooth muscle.
- *Tunica serosa* cranially – loose connective tissue covered by mesothelium.
- *Tunica adventitia* caudal to the peritoneal reflection – loose connective tissue.

Large blood vessels, venous and lymphatic plexuses and primarily sympathetic nerve bundles (derived from the pelvic plexus) and ganglia are present in the serosa and adventitia.

2.1.15.The Vestibule, Clitoris and Vulva

The following section is a summary of Priedkalns and Leiser Textbook of Histology [42] on the female reproductive system of domestic mammals.

The wall of the vestibule resembles the caudal portion of the vagina, but contains more subepithelial lymphatic nodules, especially around the clitoris. Blood vessels, venous plexuses, small lymphatic vessels and erectile cavernous tissue are abundant within the walls and become congested during oestrus.

Major vestibular glands, located in the deep part of the propria submucosa, are bilateral compound tubule-acinar glands. Their large ducts, lined by thick stratified squamous epithelium, branch into small ducts, lined by columnar mucous cells with isolated areas of goblet cells, which terminate in secretory acini, containing large mucigenous cells. They are homologous to the male bulbourethral glands, and provide mucous lubrication of the vestibule and, during coitus, possibly also the caudal vagina.

Minor vestibular glands, homologous to the male urethral glands, are scattered in the vestibular mucosa. They are bilateral small, branched, tubular mucous glands.

The clitoris consists of paired, joined erectile *Corpora cavernosa clitoridis*, covered by non-erectile, fibroelastic tissue containing a venous plexus. This is housed in a *Preputium clitoridis*. The clitoris contains numerous lymphatic nodules and sensory and autonomic nerve endings.

The vulva is formed by the labia vulvae, which are covered in skin that is richly supplied with apocrine and sebaceous glands. The hypodermis contains striated muscle fibres which form the constrictor vulvae. Numerous small blood vessels and lymphatics contained within the labia become congested during oestrus.

3. Problem Statement

- No histological studies have been done on the ovary, ligaments, uterotubal junction nor tubular structures of the reproductive tract of the female cheetah.
- Atresia of feline follicles has only been described comprehensively in the lioness.
- No feline-specific follicle classification system has been proposed.

4. Research Objectives

The purpose of this research is to:

- Accurately describe the normal histology of the ovary, ligaments and tubular parts of the reproductive tract of the female cheetah.

5. Hypothesis

- The histology of the female reproductive tract of the cheetah will resemble that of the female domestic cat.
- There may be a few histological differences between the histology of the female reproductive tract of the domestic cat and the cheetah.
- Follicular atresia in the cheetah will resemble that of the African lioness.
- Stages of folliculogenesis in the female cheetah will resemble those contemplated in the proposed feline-specific classification system (Table 8.4).

6. Benefits

- A complete histological description of the ovary, ligaments and tubular structures of the female reproductive tract of the cheetah.
- Proposal of a feline-specific follicle classification system.

7. Materials and Methods

7.1. Sample size

Seven formalin (10%) embalmed cheetah uteri and associated structures were sourced from older captive cheetahs euthanased with Pentobarbitone sodium (Euthatal, Merial Animal Health Limited) for humane reasons (6 cheetahs from Africat, Otjiwarongo, central Namibia) or presented for post-mortem (1 cheetah from the De Wildt cheetah and Wildlife Centre, North West province, South Africa). The abdominal portion of the reproductive tracts were removed during post-mortem, performed shortly after death, with a number 10 scalpel blade. It is undetermined exactly how close to the body wall the veterinarian performing the infield post-mortems transected the supporting ligaments and cervical region of the reproductive tracts.

7.2. Macro photography

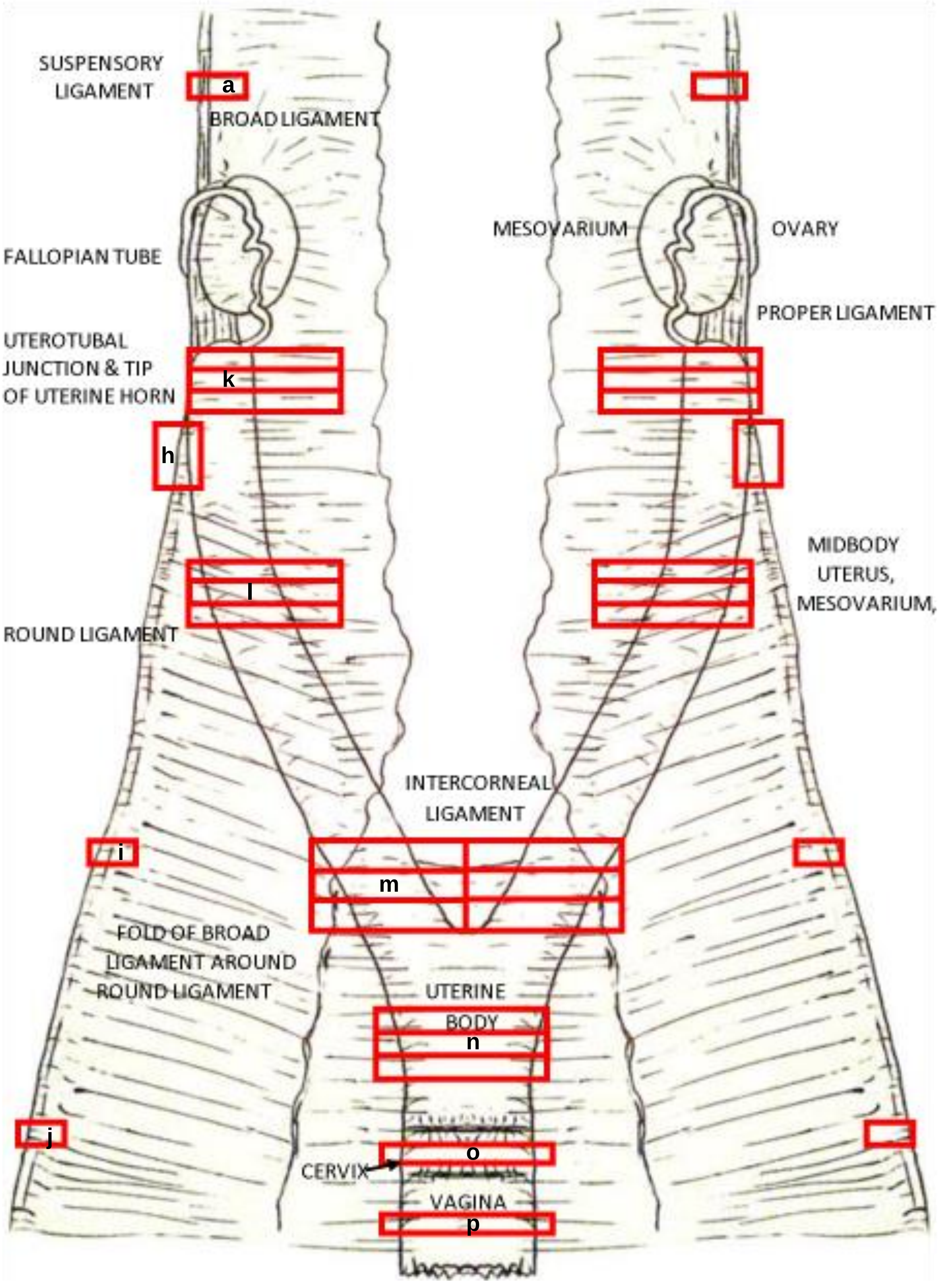
After removal of the reproductive tracts from the formalin, the specimens were photographed with a Canon 5D DSLR camera (Canon Inc, Japan) or a Huawei P8 Lite 12 MP mobile phone camera (Huawei, China) on a blue background alongside a metric ruler. Ovarian measurements were captured using the template shown (Annexure A).

7.3. Sampling

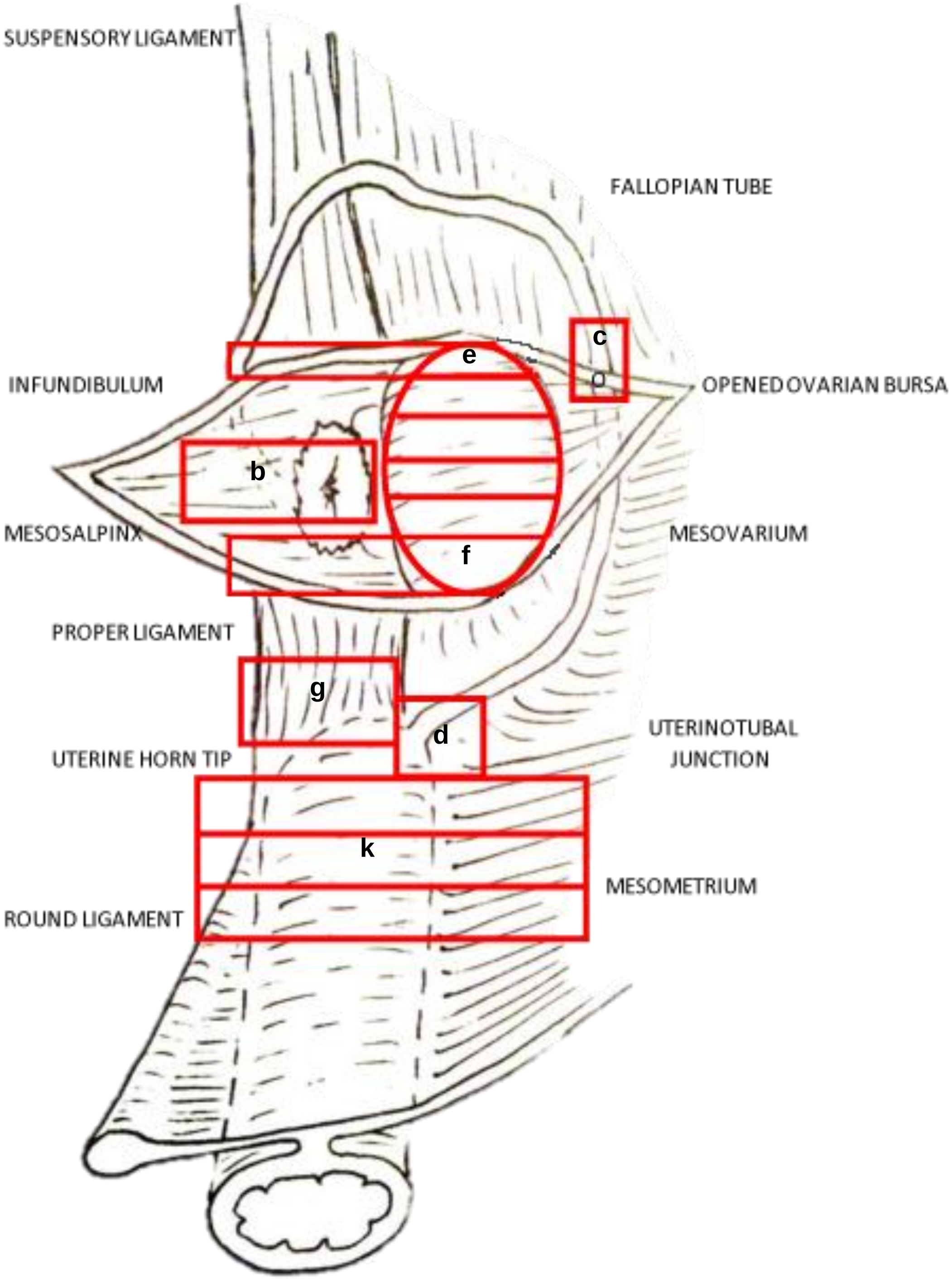
Sampling was in a transverse plane unless stated otherwise. The details of the sampling are bulleted and must be read in conjunction with the diagrams below.

- The ovaries were sectioned into 6 parts using 5 equidistant incisions in a transverse plane, taking care to not damage associated structures. The suspensory ligament was included in these samples. The right ovary of cheetah 4 & 5, and left ovary of cheetah 7 were sectioned sagittaly into 2 parts.
- The round ligament was sampled in 3 locations (reflection on uterine horn, middle third and caudal third).
- The mesometrium was examined as an extension of the associated uterine samples.
- The mesovarium and suspensory ligament was examined as a continuation of the ovarian samples.

- The uterine tube was sectioned at the infundibulum, isthmus and uterotubal junction, taking care to not damage associated structures. The right uterine tube of cheetahs 1-6, and the left infundibulum of cheetah 7 were sectioned longitudinally.
- The mesosalpinx was examined as an extension of the uterine tube samples.
- The uterus was sampled in the cranial, middle and distal thirds of the horns and at the uterine body midpoint. Three transverse, 3mm thick samples were taken from each site and placed in a non-mirrored orientation in the tissue cassettes. A 1 mm section was discarded between samples to prevent mirroring.
- The intercornual ligament was examined as an extension of the distal uterine horns.
- The cervix was sampled.
- The cranial vagina was sampled (where present) adjacent to the cervix.
- The vestibule, clitoris and vulva were not sampled as the specimens did not include these structures.


SUSPENSORY LIGAMENT
a
BROAD LIGAMENT
MESOVARIUM
OVARY
FALLOPIAN TUBE
PROPER LIGAMENT
UTEROTUBAL JUNCTION & TIP OF UTERINE HORN
k
h
MIDBODY UTERUS, MESOVARIUM,
l
ROUND LIGAMENT
INTERCORNEAL LIGAMENT
i
m
FOLD OF BROAD LIGAMENT AROUND ROUND LIGAMENT
UTERINE BODY
n
j
o
CERVIX
VAGINA
p

A


SUSPENSORY LIGAMENT
FALLOPIAN TUBE
c
e
INFUNDIBULUM
OPENED OVARIAN BURSA
b
MESOSALPINX
MESOVARIUM
f
PROPER LIGAMENT
g
d
UTERINE HORN TIP
UTERINOTUBAL JUNCTION
k
MESOMETRIUM
ROUND LIGAMENT

B

a	Cranial aspect of suspensory ligament
b	Cranial third of uterine tube & fimbriae
c	Middle part of uterine tube
d	Caudal third of uterine tube, proper ligament and uterotubal junction
e	Cranial (tubular) pole of ovary plus insertion of the suspensory ligament
f	Caudal pole of ovary plus origin of the proper ligament to mid proper ligament
g	Mid proper ligament plus tip of uterine horn (associated uterine tube removed)
h	Proximal aspect of round ligament at its point of origin from the uterine horn
i	Middle aspect of the round ligament
j	Distal aspect of round ligament near its insertion
k	Cranial third of uterine horn immediately cranial to round ligament insertion and immediately caudal to uterotubal junction
l	Middle section of uterine horn
m	Uterine horn immediately cranial to the uterine bifurcation. Includes intercornual ligament
n	Mid uterine body
o	Mid cervix
p	Cranial vaginal body

Figure 7.1: A: Diagram of the regions (excluding the ovarian region) of the reproductive tract sampled in the 7 cheetahs examined. The red rectangles represent the blocks of tissue removed for histological processing.

B: Diagram of the ovarian region sampled. The red rectangles represent the blocks of tissue removed for histological processing.

7.4. Histological sample identification

All samples were placed in tissue cassettes labelled according to Table 7.1 with a 4B pencil and engraved with a sharp utensil. The cassettes were placed in 10% formalin for transport to the laboratory.

Table 7.1: Labelling of cheetah samples. Cassettes and slides were labelled: [cheetah number (1-7) – slide number (1-53)]. Longitudinal sections: Slide numbers were suffixed with "-L" eg. 1-1-L.

Anatomical name	**Section**	**Cheetah 1**			
		Left		**Right**	
Suspensory ligament	1[a]	SLL	1-1	SLR	1-2
Uterine Tube	1[b]	UTL1	1-3	UTR1	1-6
	2[c]	UTL2	1-4	UTR2	1-7
	3[d]	UTL3	1-5	UTR3	1-8
Ovary	1[e]	OVL1	1-9	OVR1	1-14
	2	OVL2	1-10	OVR2	1-15
	3	OVL3	1-11	OVR3	1-16
	4	OVL4	1-12	OVR4	1-17
	5	OVL5	1-13	OVR5	1-18
Proper ligament	1[f]	PLL1	1-50	PLR1	1-52
	2[g]	PLL2	1-51	PLR2	1-53
Round ligament	1[h]	RLL1	1-19	RLR1	1-22
	2[i]	RLL2	1-20	RLR2	1-23
	3[j]	RLL3	1-21	RLR3	1-24
Uterine Horn	1[k]	UHL1.1	1-25	UHR1.1	1-34
		UHL1.2	1-26	UHR1.2	1-35
		UHL1.3	1-27	UHR1.3	1-36
	2[l]	UHL2.1	1-28	UHR2.1	1-37
		UHL2.2	1-29	UHR2.2	1-38
		UHL2.3	1-30	UHR2.3	1-39
	3[m]	UHL3.1	1-31	UHR3.1	1-40
		UHL3.2	1-32	UHR3.2	1-41
		UHL3.3	1-33	UHR3.3	1-42
Uterine Body	1[n]	UB1.1	1-43		
		UB1.2	1-44		
		UB1.3	1-45		
Cervix	1[o]	CE	1-46		
Vagina	1[p]	VA	1-47		

a	Cranial aspect of suspensory ligament
b	Cranial third of uterine tube & fimbriae
c	Middle part of uterine tube
d	Caudal third of uterine tube, proper ligament and uterotubal junction
e	Cranial (tubular) pole of ovary plus insertion of the suspensory ligament
f	Caudal pole of ovary plus origin of the proper ligament to mid proper ligament
g	Mid proper ligament plus tip of uterine horn (associated uterine tube removed)
h	Proximal aspect of round ligament at its point of origin from the uterine horn
i	Middle aspect of the round ligament
j	Distal aspect of round ligament near its insertion
k	Cranial third of uterine horn immediately cranial to round ligament insertion and immediately caudal to uterotubal junction
l	Middle section of uterine horn
m	Uterine horn immediately cranial to the uterine bifurcation. Includes intercornual ligament
n	Mid uterine body
o	Mid cervix
p	Cranial vaginal body

7.5. Processing

The following routine histological methods were used to prepare the samples, embed them in wax, section, stain with haematoxylin and eosin (H&E) and mount them on glass slides labelled with a pencil.

At the laboratory the cassettes were removed from the 10% formalin with stainless steel forceps and placed into a Histo-Tek VP1 vacuum infiltration tissue processor (Sakura Finetek, USA) which replaces the water in the tissues with paraffin. Thereafter the processed cassettes were transferred to the paraffin cassette storage area of a Histo-Center 11-N wax embedding machine (Sybron, USA) for wax embedding as follows. A cassette was removed from the paraffin tank and the cassette cover was removed. A suitable sized stainless steel base mould for embedding the specimens in wax was selected from the hot plate storage compartment The base mould was placed under the liquid wax dispenser and a small amount of liquid Pathwax (Cell Path Services CC, South Africa) was dispensed to cover the bottom of the base mould. A metal forceps was used to transfer the specimens to the base mould where the specimen was orientated with the cut surface facing down. The base mould was placed on the cold plate to allow the wax to start solidifying. Once this started and the specimen was stuck in the correct orientation in the base mould, but before the wax was all solidified, more liquid wax was added from the wax dispenser to fill the base mould. The empty labelled plastic cassette was then pushed onto the top of the base mould and the wax allowed to solidify. This process was repeated for all the specimens. The solid wax cassettes were then separated from the base moulds and a blunt knife used to scrape off excess wax from the sides of the cassettes. The wax blocks were then sectioned with a Shandon finesse 325 microtome machine (Thermo Scientific, UK) using a Feather microtome blade S35 (Feather Safety Razor Co. Ltd., Japan). The surface sections were discarded until a uniform wax block surface was achieved. Six separate sections were cut, one after the other, were floated on water to allow them to flatten and then transferred to a Histoband+ adhesive microscope slide (Marienfeld, Germany). The glass slide was identified with a pencil according to the cassette labelling. The slide was then placed in an oven (Labotec, South Africa) at 60°C for 15-20 minutes. The slide was removed from the drying oven. The affixed specimens then underwent a manual dewaxing and staining process as follows:

- The slide was dewaxed in a closed container of xylene for 5 minutes.
- Hydration of the slide specimen through graded alcohols:
    a. Rinse the slide in 100% alcohol for 2 minutes.
    b. Rinse the slide in 96% alcohol for 2 minutes.
    c. Rinse the slide in 70% alcohol for 2 minutes.
- Wash the slide in distilled water for 2 minutes.
- Immerse the slide in Lily Mayer’s Haematoxylin for 10 minutes.
- Rinse thoroughly in distilled water until no traces of haematoxylin are coming out.
- Differentiate quickly in alcohol
- Leave in gently running tap water for 8 minutes to blue.
- Rinse in 70% alcohol for 2 minutes.
- Stain in Eosin for 3 minutes
- Dehydrate in graded alcohols
    d. Rinse the slide in 96% alcohol for 2 minutes.
    e. Rinse the slide in 96% alcohol for 2 minutes.
    f. Rinse the slide in 100% alcohol for 3 minutes.
- Rinse the slide in xylene for 1-2 minutes.

The slides were then taken to the extractor fan for mounting, ensuring the fan had been on for two minutes prior to use. Two drops of Entellan was used to affix a protective glass cover slip over the stained mounted specimens. A clean Kleenex tissue was used to wipe off the excess Entellan.

7.6. Examining

The samples were examined and relevant structures photographed at 40x (200µm), 100x (100µm), 200x (50 µm) 400x (20µm) and 1000x (10µm) magnifications (microbar measurements are indicated in the brackets) using an Olympus BX63 light microscope (Olympus corporation, Japan) with bright field illumination. Images were captured using an Olympus DP72 camera. The CellSens Dimension software package (Olympus corporation, Japan) was used to adjust sharpening, contrast and brightness as needed. The Photoshop software package (Adobe, USA) was used to further adjust white balance, merge photomicrographs and make other minor corrections as needed. Microbar information was burned into the images, and the images were studied and annotated appropriately.

8. Results

8.1. General anatomical features of the female reproductive tract

The female reproductive tract (Figure 8.1) consisted of bilateral suspensory ligaments with ovaries attached to their free margin (Figure 8.2). The suspensory ligament merged with the proper ligament which merged with the tip of the uterine horn (Figure 8.2). The suspensory ligament split into two parts at the level of the tubal extremity of the ovary. One part ran in the mesosalpinx to the junction of the isthmus with the external surface of the uterine horn, while the other was continuous with the hilus of the attached ovary and merged with the proper ligament (Figure 8.10 F).

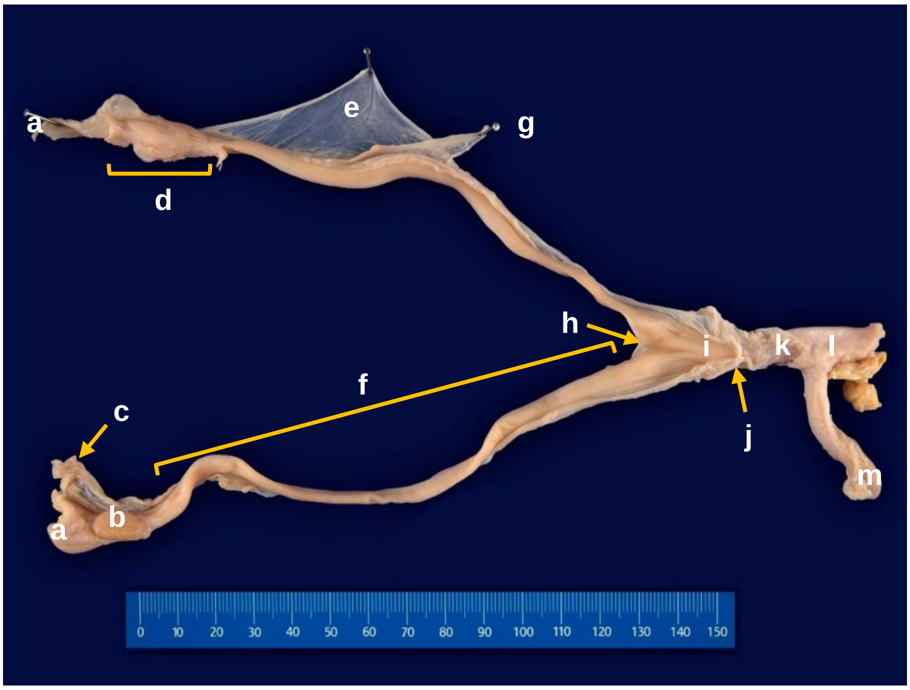


Figure 8.1: Gross anatomy of the female reproductive tract (ventral aspect) of a 10.5 year-old cheetah. Cranial is left. Suspensory ligament (a); Right ovary (b) with the ovarian bursa retracted (c); Left ovary covered by the ovarian bursa (d); Broad ligament (e) which has been partially reflected. It is retracted against the uterine horns, uterine body, ovary and suspensory ligament in the rest of the photograph and is therefore not visible; Right uterine horn (f); Left round ligament (g).The round ligament has not been reflected on the opposite horn; Intercornual ligament (h); Uterine body (i); Peritoneal reflection (j), Cervix (k); Vagina (l); Colon (m) which has been reflected.

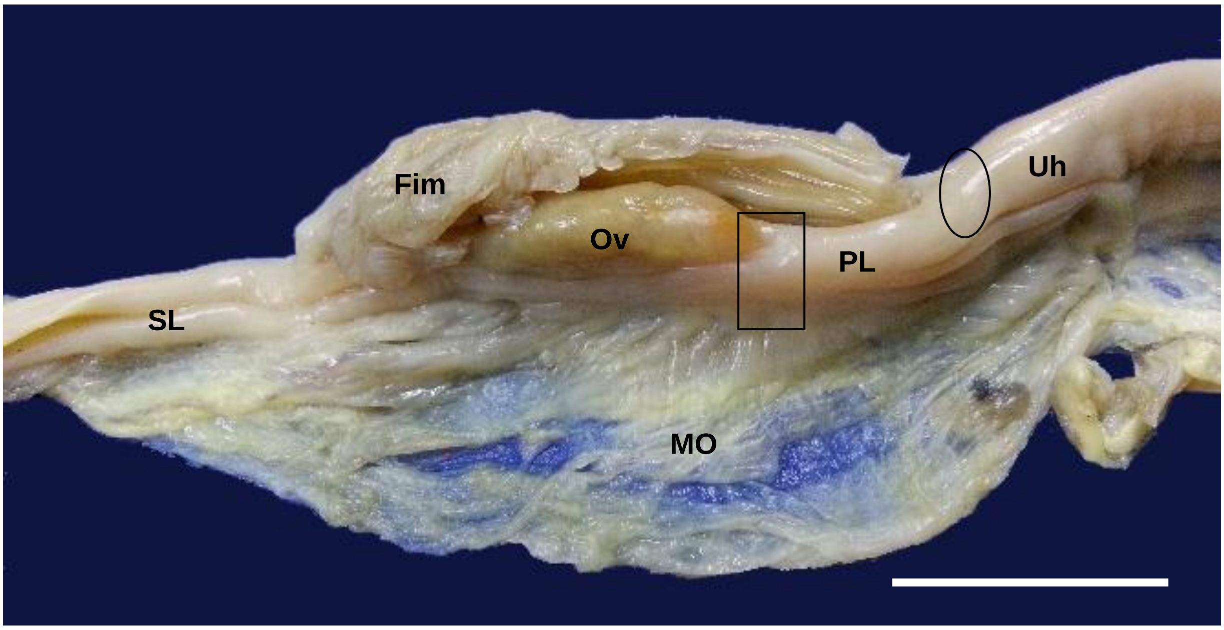


Figure 8.2: Medial view of the left ovary (Ov) of a 10.5 year-old cheetah. Cranial is left. The ovary is attached to the free margin of the suspensory ligament (SL). The suspensory ligament is continuous (black rectangle) with the proper ligament (PL) which attaches (black oval) to the uterine horn; Fimbriae (Fim); Uterine horn (Uh); Mesovarium (MO). Bar = 10 mm.

A prominent band of tissue, referred to as the ‘uterine sling’ in this dissertation, continued distally from the proper ligament in the attachment of the broad ligament to the uterine horns (Figure 8.3), giving off the round ligament in the proximal third of the uterine horn (Figure 8.4), flanking the uterine body (Figure 8.5) and finally terminating at the midpoint of the uterine body or on the cervix (Figure 8.6).

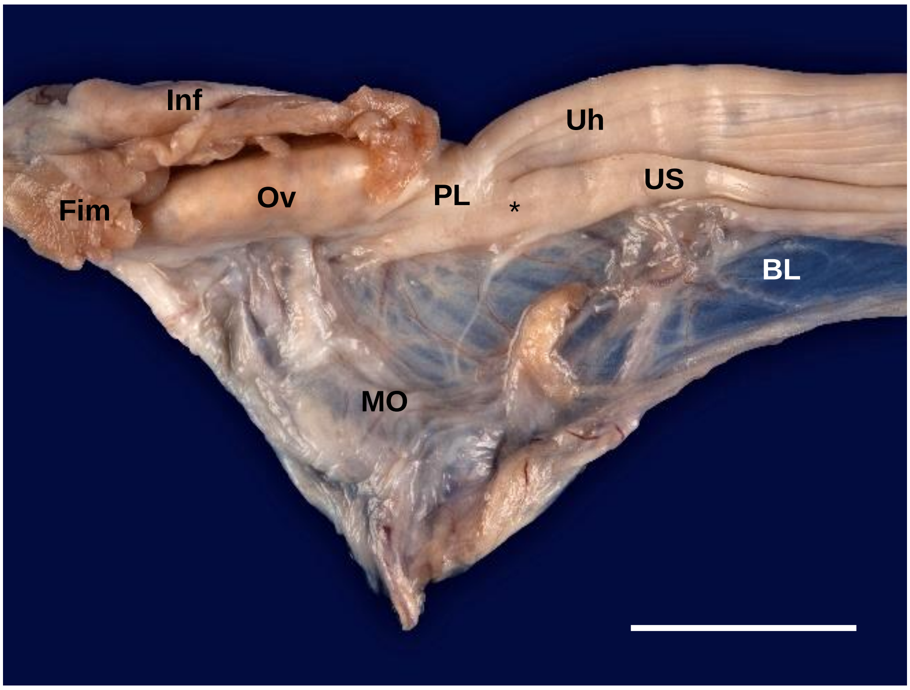


Figure 8.3: Right medial aspect of the continuation (*) of the proper ligament (PL) with the uterine sling (US) in a 10.5 year-old cheetah. Cranial is left. Uterine horn (Uh); Ovary (Ov); Infundibulum (Inf); Mesovarium (MO); Broad ligament (BL); Fimbriae (Fim). Bar = 10 mm.

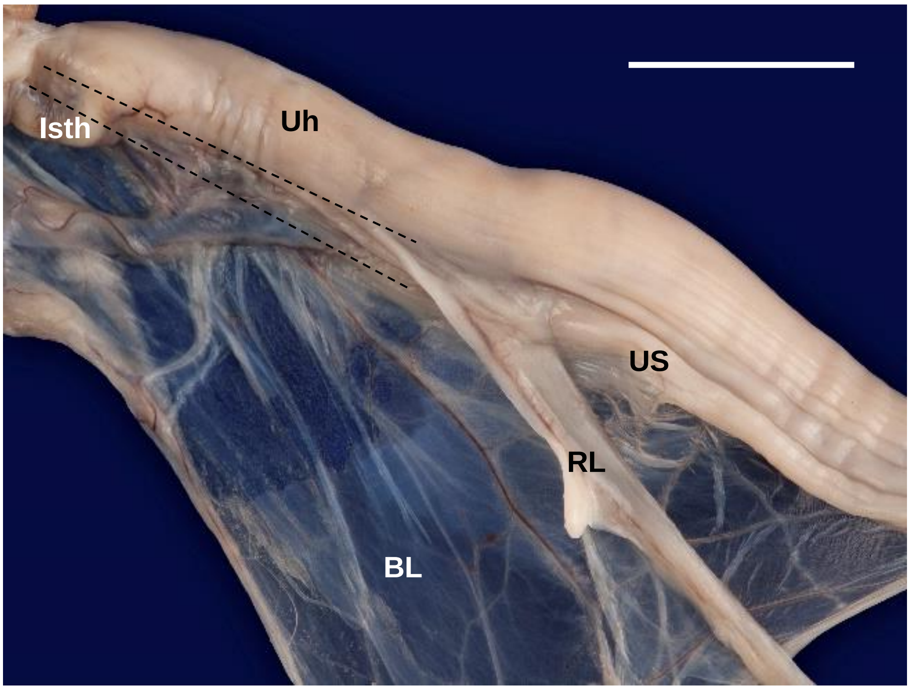


Figure 8.4: Lateral view of the left uterine horn (Uh) of a 10.5 year-old cheetah. Cranial is left. The uterine sling (US) is hidden cranially by the tip of the uterine horn and broad ligament, and is best viewed from the medial aspect (see Figure 8.3). The black dotted lines indicate the position of the hidden cranial part of the uterine sling. Isthmus (Isth); Round ligament (RL); Broad ligament (BL). Bar = 10 mm.

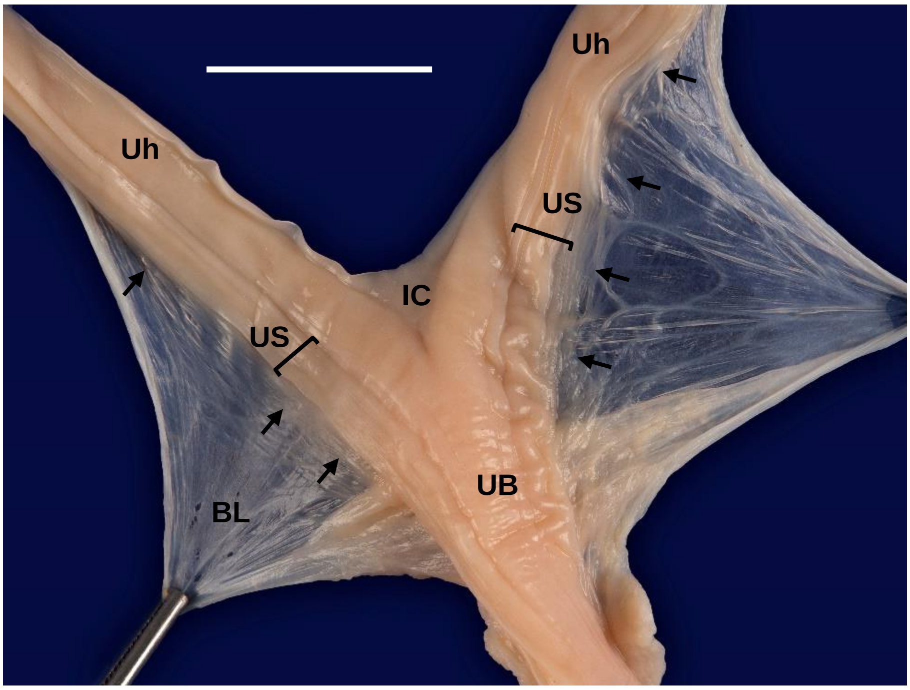


Figure 8.5: Ventral view of the uterine sling (US) in the region of the uterine body (UB) and junction of the uterine horns (Uh) of a 10.5 year-old cheetah. Cranial is left. The uterine sling is substantial and its strands (arrows) blend into the broad ligament (BL). Uterine body (UB); Intercornual ligament (IC). Bar = 19 mm.

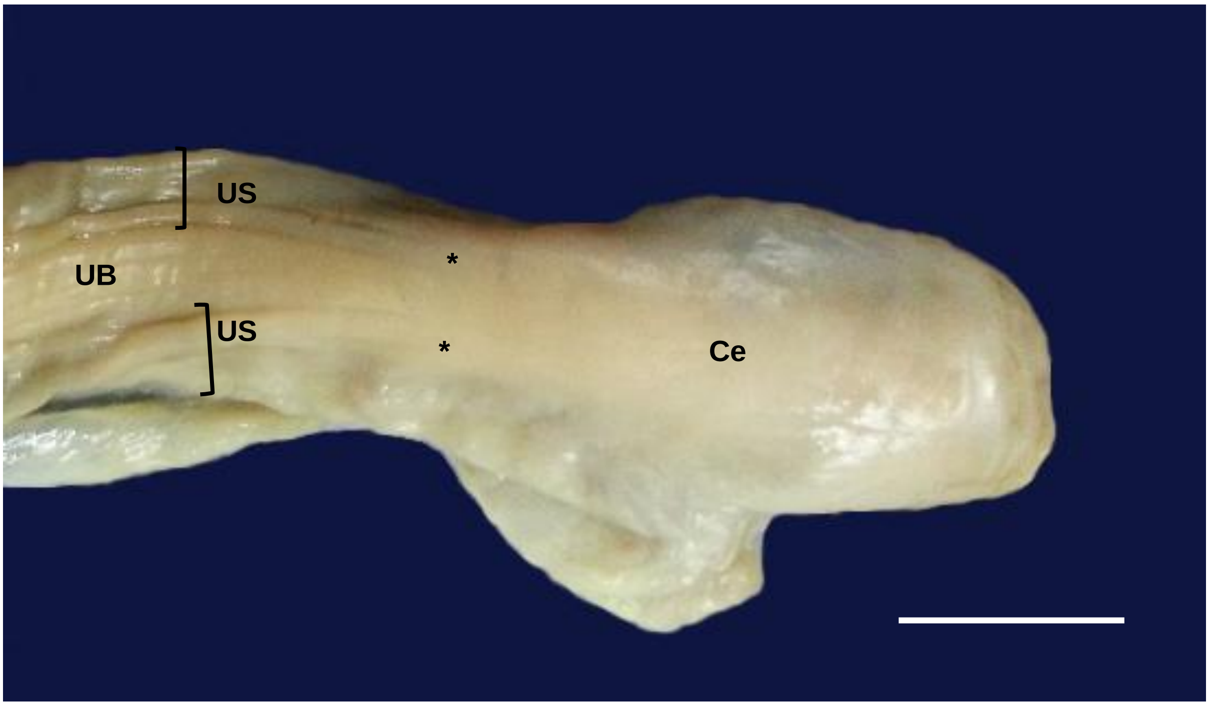


Figure 8.6: Ventral view of the termination (*) of the uterine sling (US) at the cervix (Ce) of a 10.5 year-old cheetah. Cranial is left. Uterine body (UB). Bar = 10 mm.

The ovaries varied in size (Table 8.1) and were larger in the two younger cheetahs, aged 3 and 7 years, compared to the older cheetahs. The ovaries were covered by the ovarian bursa which opened medially and covered the free margin and lateral ovarian surfaces. It formed a clear window laterally through which the ovary could be visualised. The mesosalpinx contained the uterine tube which ran from the abdominal ostium to the tip of the uterine horn (Figure 8.7).

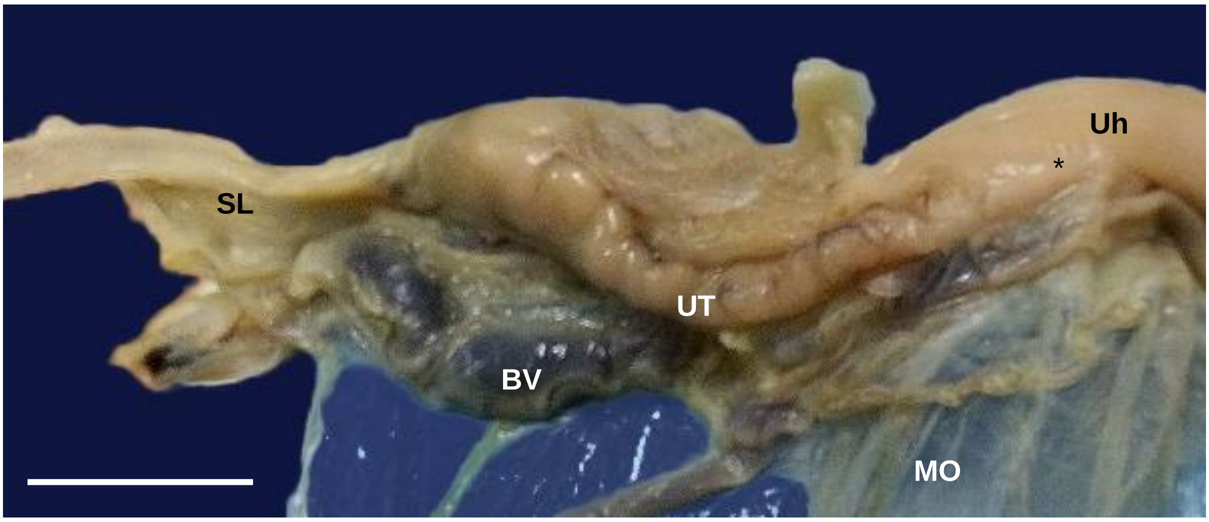


Figure 8.7: Lateral aspect of the right ovary of a 10.5 year-old cheetah covered by the mesosalpinx containing the uterine tube (UT). Cranial is left. The uterine tube enters the tip of the uterine horn (*) on the mesometrial margin of the uterine horn. Suspensory ligament (SL); Mesovarium (MO); Tip of the uterine horn (Uh); Ovarian blood vessels (BV). Bar = 10 mm.

Table 8.1: Gross dimensions of the formalinised cheetah ovaries in millimetres.

Cheetah id	**Ovary Length**		**Width (medial to lateral surface)**		**Height (mesovarium to free margin)**		**Age**
	Left	**Right**	**Left**	**Right**	**Left**	**Right**	**yr**
1	17	17	8	10	5	4	3
2	11	9	7	5	4	5	10.5
3	11	8	4	6	5	5.5	10
4	11	14	6	5	3	3	10
5	13	12	6	6	4	4	10
6	10	13	6	7	5	3	10.5
7	19	19	10	10	5	4	7

The uterine horn was cylindrical and gradually increased in diameter from its tip to its junction with the uterine body where the contralateral horn similarly joined the uterine body. The uterine horns were connected at their base by a thin intercornual ligament (Figure 8.5) and ran a short distance parallel to each other in the uterine body separated by the uterine velum before joining. This was only observable on cross-section of the uterine body (Figure 8.83).

A thick cervix separated the uterus from the vagina (Figure 8.6; Figure 8.85). The broad ligament was present from the suspensory ligament to the cervix and contained large blood vessels (Figure 8.21), thereafter the caudal reproductive tract ran retroperitoneally.

8.2. Ligaments of the female reproductive tract

Five macroscopically discernible ligaments were present in the female cheetah reproductive tract. They were the suspensory ligament, proper ligament, round ligament, intercornual ligament and broad ligament. The broad ligament was further identified according to the region of the reproductive tract it suspended as the mesovarium, mesosalpinx or mesometrium.

8.2.1. Suspensory ligament

Macroscopically the suspensory ligament was formed of 2 contiguous components, a main component and secondary component. The main component formed a single, discreet, flat cord that was continuous with the proper ligament. The ovary attached to the free margin of the main component of the suspensory ligament. The secondary component the main component and was visible as a separate, flat cord in the mesovarium. The secondary component joined the mesovarial margin of the main component opposite the cranial third of the ovary. The thinner component was poorly developed unilaterally on the left side in 2 cheetahs (cheetahs 1&7)(Figure 8.8) and well develop in in 5 of 7 cheetahs (cheetahs 2-6)(Figure 8.9).

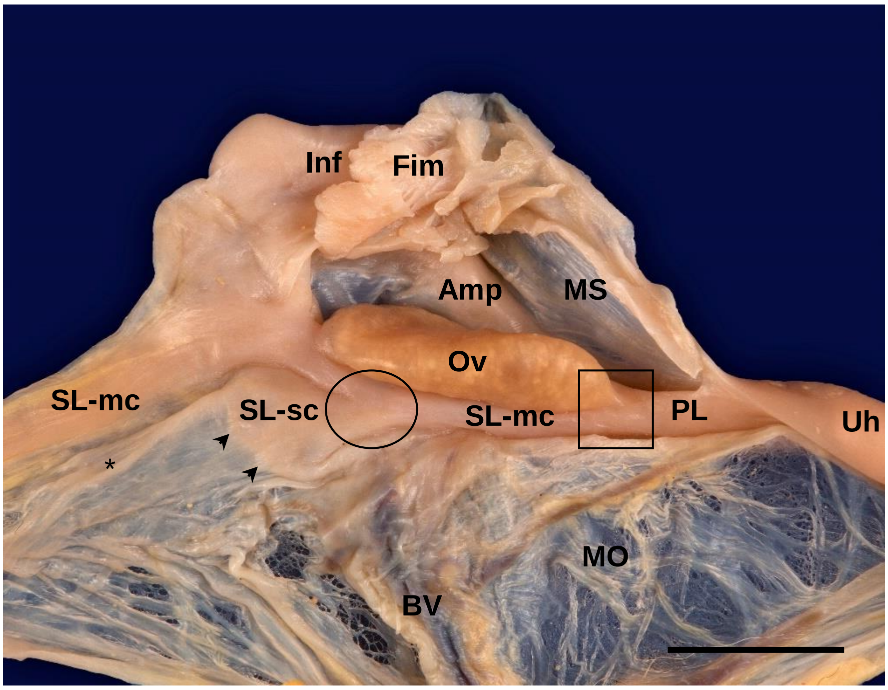


Figure 8.8: Association between the ligaments and left ovarian structures to each other in a 3 year-old cheetah (medial view). Cranial is left. The suspensory ligament secondary component (SL-sc) is poorly developed but some strands (*) are seen running in the mesovarium (MO). The remainder of secondary component terminates abruptly in the mesovarium (arrow heads). The secondary component joins (oval) the main component (SL-mc) opposite the cranial third of the ovary (Ov). The main component is continuous with the proper ligament (PL) in the area outlined by the square. Tip of uterine horn (Uh); Ovarian vascular supply (BV); Infundibulum (Inf); Fimbriae (Fim); Ampulla of the uterine tube (Amp); Mesosalpinx (MS). Bar = 10 mm.

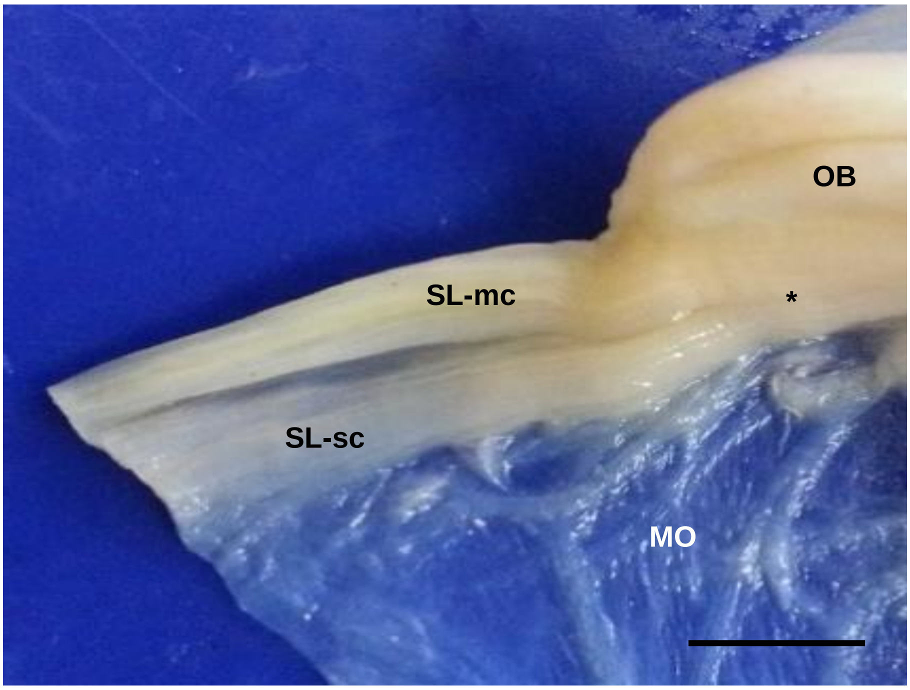


Figure 8.9: The left suspensory ligament of cheetah 4 age 10 yr (lateral view). The portion on the left has been cut. Cranial is left. A distinct, strap like secondary component of the suspensory ligament (SL-sc) is present and merges (*) with the main component (SL-mc) opposite the cranial third of the ovary which is obscured by the ovarian bursa (OB). Mesovarium (MO). Bar = 3 mm.

In 5 of 6 cheetahs (cheetahs 2 & 4-7), the suspensory ligament was observed to send off a side branch between the tubal extremity of the ovary and the ovarian midpoint that ran in the mesosalpinx (Table 8.2). One of six cheetahs had no side branches (cheetah 3)(Figure 8.10 A). In one of six cheetahs the side branch terminated in the mesosalpinx (cheetah 4) and appears to be an anomaly (Figure 8.10 B). In 4 of 6 cheetahs (cheetahs 2 & 5-7) the suspensory ligament side branch joined the uterine horn where the uterine tube entered the myometrium on the tip of the uterine horn in (Figure 8.10 C-F). One cheetah had one side branch on the left and none on the right of the reproductive tract (cheetah 4). Two cheetahs had a side branch only on the right side of the reproductive tract (cheetahs 2 & 4). Three cheetahs had a side branch which originated from both the main component of the left suspensory ligament in the region of the tubal extremity of the ovary and from the left suspensory ligament main component opposite the mid-point of the ovary (cheetahs 2, 6-7)(Figure 8.10 C&F). The suspensory ligament side branch was not mirrored in any cheetahs by the contralateral suspensory ligament side branch which showed its own individual variation within the same cheetah (cheetahs 2, 4-7) (Table 8.2; Figure 8.10 A-B, D-E).

Table 8.2: Comparison of the presence, origin and termination of the side branch of the suspensory ligament. (*) Indicates the origin of the suspensory ligament branch. (✢) Indicates the insertion of the suspensory ligament branch. (Blue shading) indicates cheetahs with no suspensory ligament branch. Cheetah 1 – no data was recorded.

Cheetah		Origin		Termination	
	Horn	Tubal extremity of the ovary	Middle of the ovary	Mesosalpinx	Isthmus’ external junction with the uterine horn
2	Right	*			✢
(10.5 yr)	Left	*	*		✢
3	Right				
(10 yr)	Left				
4	Right				
(10 yr)	Left	*		✢	
5	Right	*			✢
(10 yr)	Left		*		✢
6	Right	*			✢
(10.5 yr)	Left	*	*		✢
7	Right	*			✢
(7 yr)	Left	*	*		✢

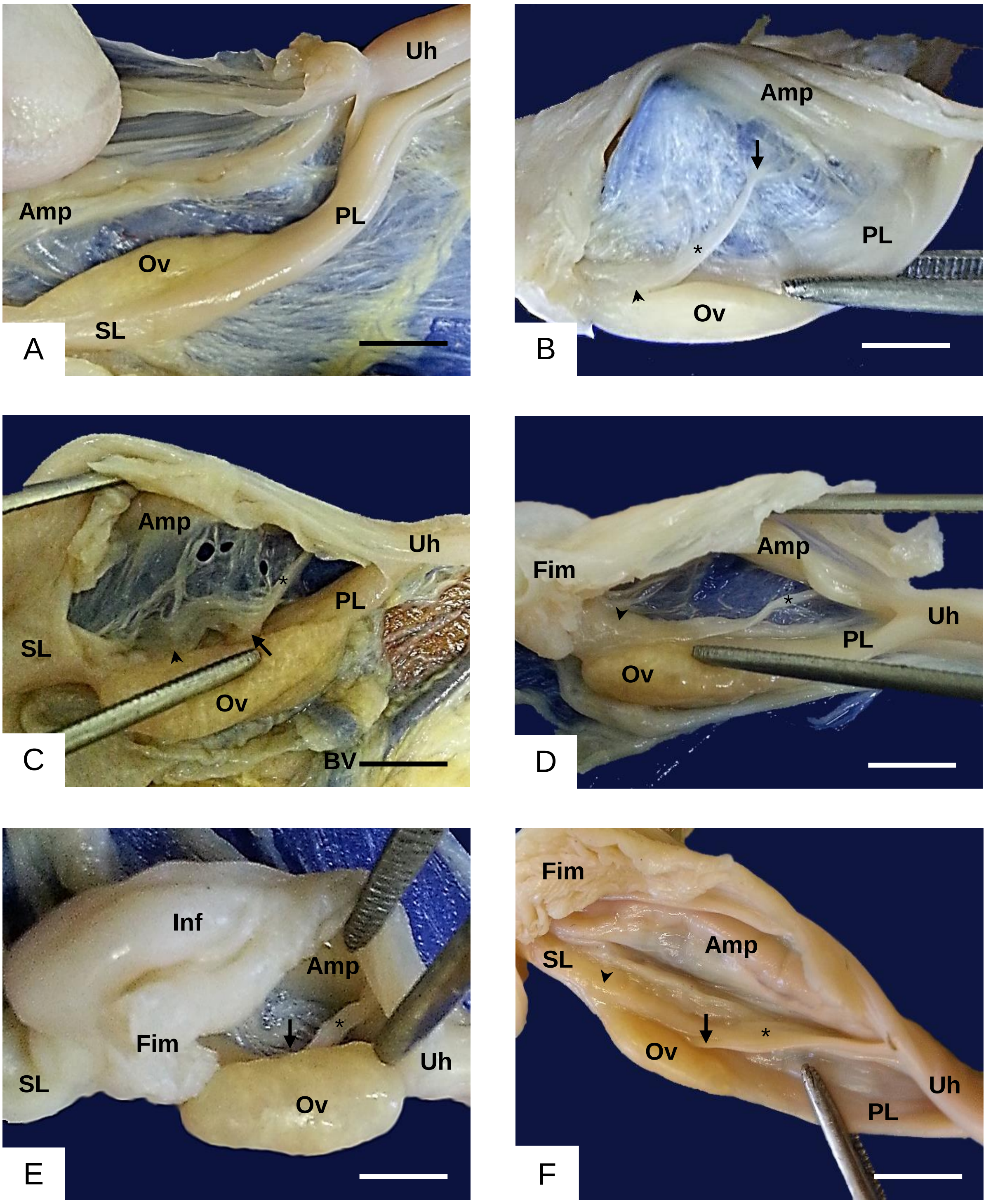


Figure 8.10: Variations in the presence and development of the suspensory ligament side branch. The ovarian bursa has been reflected in all the images. Cranial is left in all the images. All images are ventro-lateral views.

A: No side branch is present. Proper ligament (PL); Ampulla (Amp); Ovary (Ov); Tip of uterine horn (Uh); Suspensory ligament (SL) *.

B: The side branch (*) originates from the tubal extremity (arrow head) of the suspensory ligament and terminates in the mesosalpinx at the tip of the arrow. Proper ligament (PL); Ampulla (Amp); Ovary (Ov) **.

C: A thin, fused side branch (*) is present originating from the tubal extremity (arrow head) and mid ovarian region (arrow) of the suspensory ligament. Proper ligament (PL); Ampulla (Amp); Ovary (Ov); Tip of uterine horn (Uh); suspensory ligament (SL); Ovarian vascular supply (BV) ***.

D: A thin side branch (*) is present originating from the tubal extremity (arrow head) of the suspensory. Proper ligament (PL); Ampulla (Amp); Ovary (Ov); Tip of uterine horn (Uh); Fimbriae (Fim) ^.

E: The side branch (*) originates from the mid ovarian region (arrow) of the suspensory. Ampulla (Amp); Ovary (Ov); Tip of uterine horn (Uh); Infundibulum (Inf); Suspensory ligament (SL); Fimbriae (Fim) ^^.

F: The main component of the left suspensory ligament is continuous with the proper ligament (PL) at the uterine end of the ovary (Ov) shown by the black oval. A thick, fused side branch of the suspensory ligament is present originating from the tubal extremity (arrow head) and mid ovarian region (arrow) of the suspensory ligament. Ampulla (Amp); Fimbriae (Fim); Tip of uterine horn (Uh) ^^^.

* 10 year-old cheetah; right ovary. Bar = 3 mm.

** 10 year-old cheetah; left ovary. Bar = 4 mm.

*** 7 year-old cheetah; left ovary. Bar = 6 mm.

^ 10 year-old cheetah; right ovary. Bar = 5 mm.

^^ 10 year-old cheetah; left ovary. Bar = 5 mm.

^^^ 10.5 year-old cheetah; left ovary. Bar = 6 mm.

Histologically the suspensory ligament main component, secondary component and side branch consisted of longitudinally orientated smooth muscle fibres separated by dense, irregular connective tissue into distinct bundles (Figure 8.11). Small blood vessels were present in the connective tissue (Figure 8.12). The suspensory ligament was covered by peritoneal mesothelium. The main component of the suspensory ligament continued the length of the ovary sending off smooth muscle fibres that infiltrated the ovarian medulla via the hilus. At the uterine extremity of the ovary it was continuous with the proper ligament and no distinction could be made between the two ligaments (Figure 8.14). *Rete ovarii* and large blood vessels supplying the ovary traversed the suspensory ligament in the hilus region of the ovary.

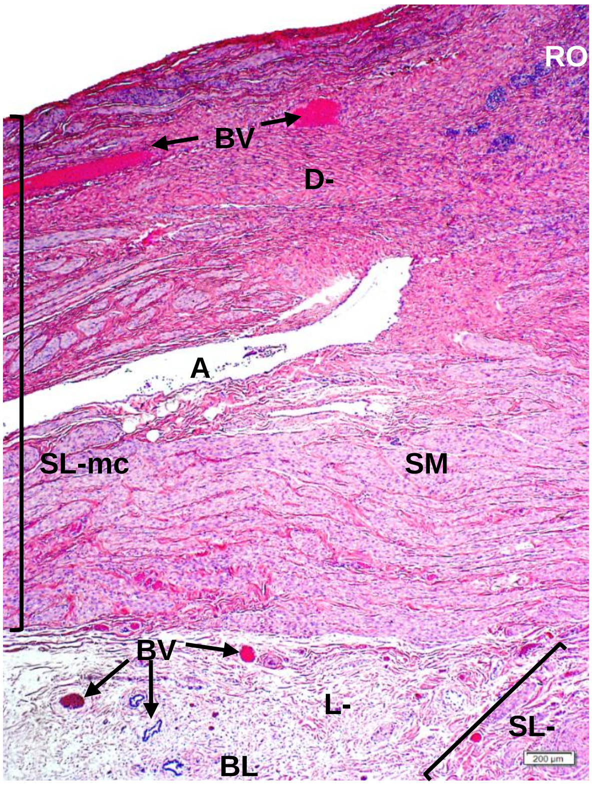


Figure 8.11: Longitudinal section of the main component of the suspensory ligament (SL-mc) in the region of the tubal extremity of the ovary. Dense connective tissue (D-ct) separates the smooth muscle (SM) into bundles. The white strip (A) represents an artefact. *Rete ovarii* (RO); Loose connective tissue (L-ct); Broad ligament (BL); Blood vessels (BV); Suspensory ligament – secondary component (SL-tc). Bar = 200 µm.

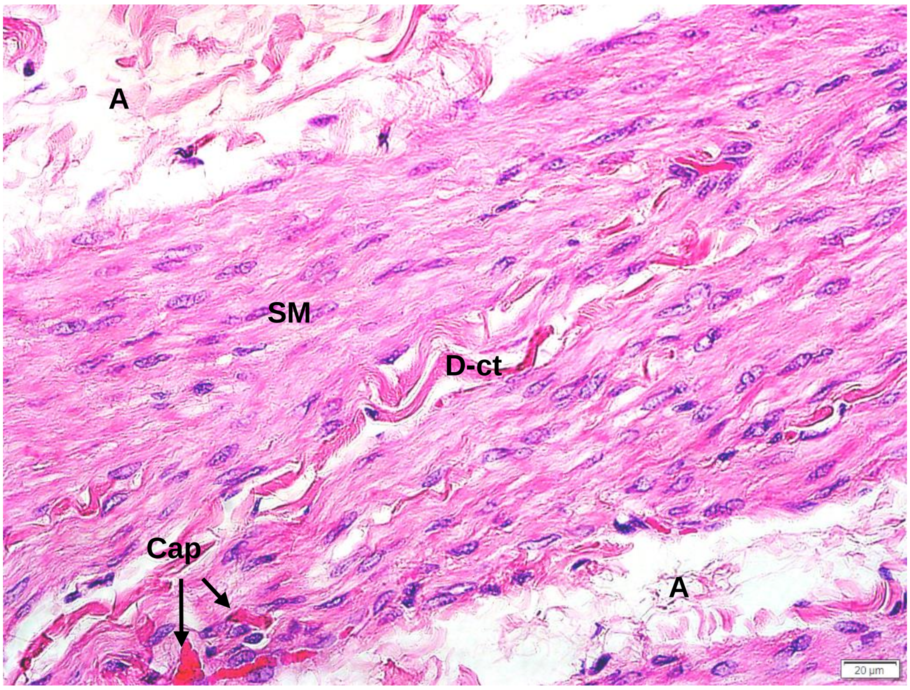


Figure 8.12: Longitudinal section of the suspensory ligament main component. Tearing of the tissues occurred (A) during processing Smooth muscle (SM); Dense, irregular connective tissue (D-ct). Capillaries (Cap). Bar = 20 µm.

8.2.2. Proper ligament

The proper ligament appeared macroscopically to consist of a single cord stretching from the uterine pole of the ovary to the tip of the uterine horn. In 2 of 6 cheetahs (cheetahs 2&4) the proper ligament formed three longitudinal folds (Figure 8.13 A&C) at the insertion on the tip of the uterine horn. In two of six cheetahs (cheetahs 6&7) the proper ligament displayed folding at the insertion on the uterine horn unilaterally while the contralateral proper ligament insertion showed no folding. In 1 of 6 cheetahs (cheetah 7) the proper ligament formed two longitudinal folds on the left insertion and no longitudinal folds on the right insertion (Figure 8.13 B). In one cheetah (cheetah 1) data was only captured for the left proper ligament which displayed no folding at the insertion (Figure 8.13 D). In 1 of 6 cheetahs (cheetah 3) the proper ligament formed four longitudinal folds on the left insertion and 3 longitudinal folds on the right insertion. In 1 of 6 cheetahs (cheetah 5) the proper ligament formed three longitudinal folds on the left insertion and two longitudinal folds on the right insertion. In 1 of 6 cheetahs (cheetah 6) the proper ligament formed three longitudinal folds on the left insertion and no longitudinal folds on the right insertion (Table 8.3).

Table 8.3: Comparison of the number of folds present in the proper ligament at the tip of the uterine horn. - No data was captured for cheetah 1 right proper ligament.

Cheetah id	**Proper ligament insertion folds**		**Age**
	Left	**Right**	**yr**
1	0	-	3
2	3	3	10.5
3	4	3	10
4	3	3	10
5	3	2	10
6	3	0	10.5
7	2	0	7

The proper ligament originated as a continuation of the suspensory ligament at the uterine pole of the ovary and was histologically indistinguishable from the suspensory ligament (Figure 8.14). The proper ligament was composed of smooth muscle bundles surrounded by, dense, regular connective tissue (Figure 8.15 A). Part of the proper ligament terminated on the tip of the uterine horn, where the longitudinal smooth muscle fibres blended with the outer longitudinal smooth muscle layer of the uterine horn (Figure 8.15 B). The rest of the proper ligament continued as the uterine sling (Figure 8.13 C)

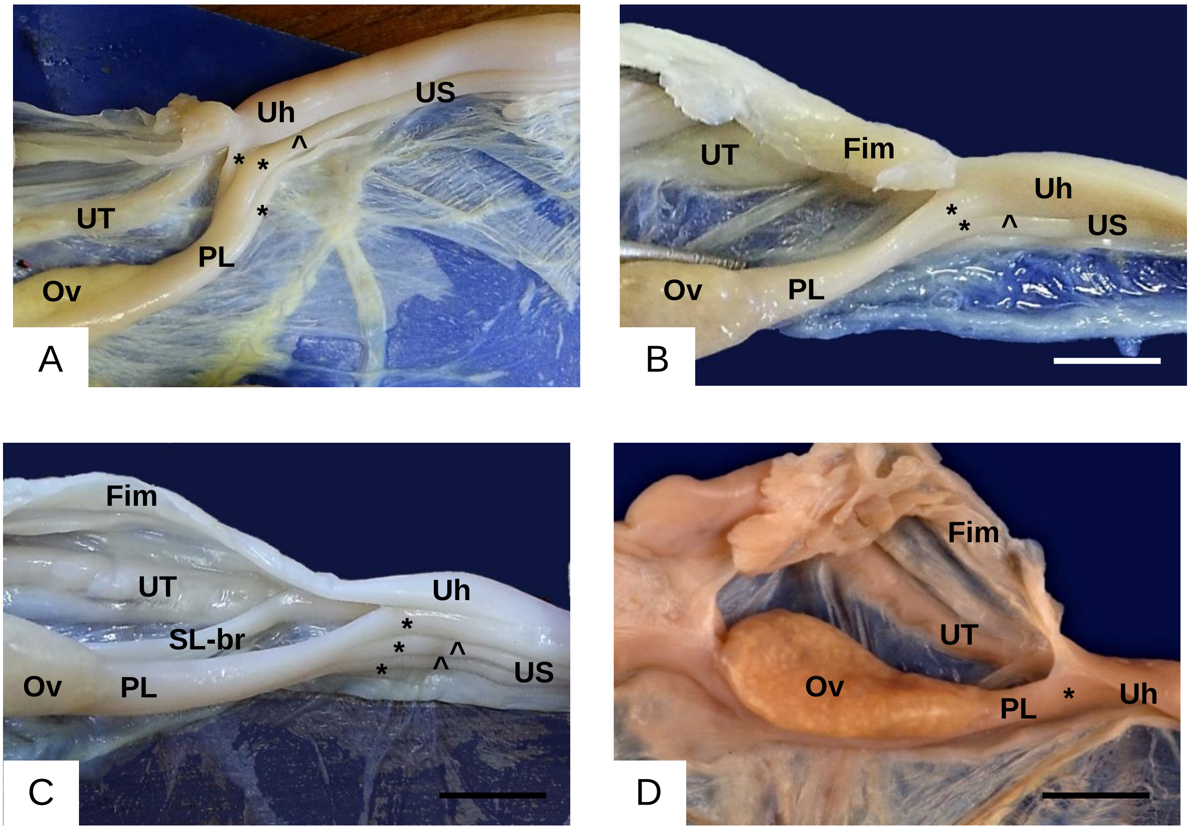


Figure 8.13: Macroscopic photographs showing variations in the appearance of the termination of the proper ligament. All images are medial view. Cranial is left.

A: Three folds (*) are present in the termination of the proper ligament, the central fold (^) is continuous with the uterine sling (US). Tip of Uterine horn (Uh); Ovary (Ov); Uterine tube (UT); Proper ligament (PL); Folds of the proper ligament (*) *.

B: Two folds (*) are present in the termination of the proper ligament, one fold (^) is continuous with the uterine sling (US). Ovary (Ov); Uterine tube (UT); Proper ligament (PL); Fimbriae (Fim); Tip of Uterine horn (Uh) **.

C: Three folds (*) are present in the termination of the proper ligament, two folds (^) are continuous with the uterine sling (US). Ovary (Ov); Uterine tube (UT); Suspensory ligament side branch (SL-br); Fimbriae (Fim); Uterine horn (Uh) ^.

D: No folds (*) are present in the termination of the proper ligament (PL). Ovary (Ov); Uterine tube (UT); Fimbriae (Fim); Uterine horn (Uh) ^^.

* 10 year-old cheetah; right proper ligament. Bar = 10 mm.

** 10 year-old cheetah; right proper ligament. Bar = 6 mm.

^ 10.5 year-old cheetah; left proper ligament. Bar = 8 mm.

^^ 3 year-old cheetah; left proper ligament. Bar = 8 mm.

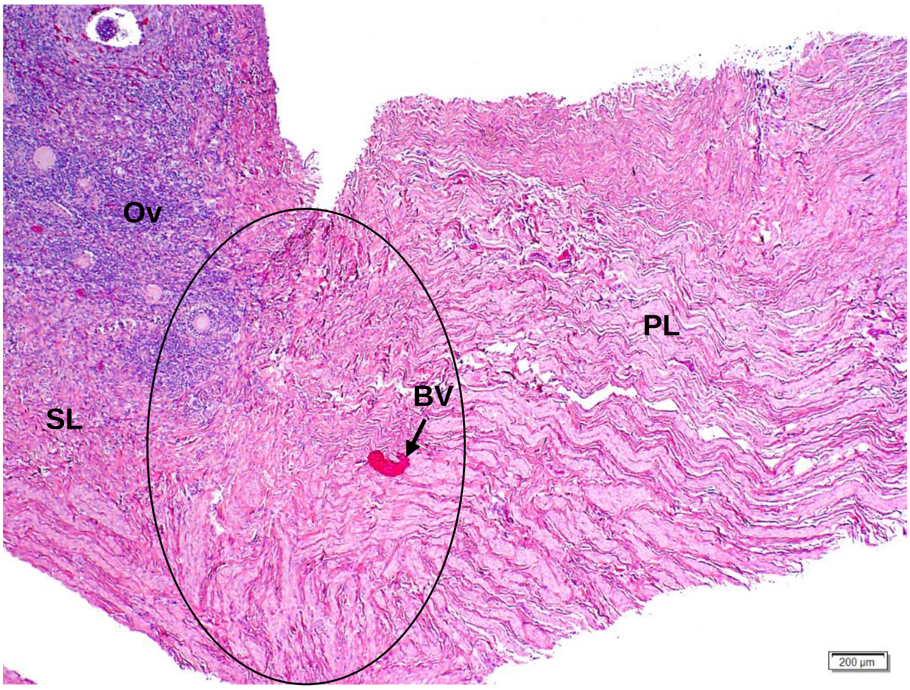


Figure 8.14: Longitudinal section through the origin of the proper ligament at the uterine pole of the ovary (Ov). There is no distinction between the origin of the proper ligament and termination of the suspensory ligament (black oval). Smooth muscle of the proper ligament (PL); Suspensory ligament (SL); Blood vessel (BV). Bar = 200 µm.

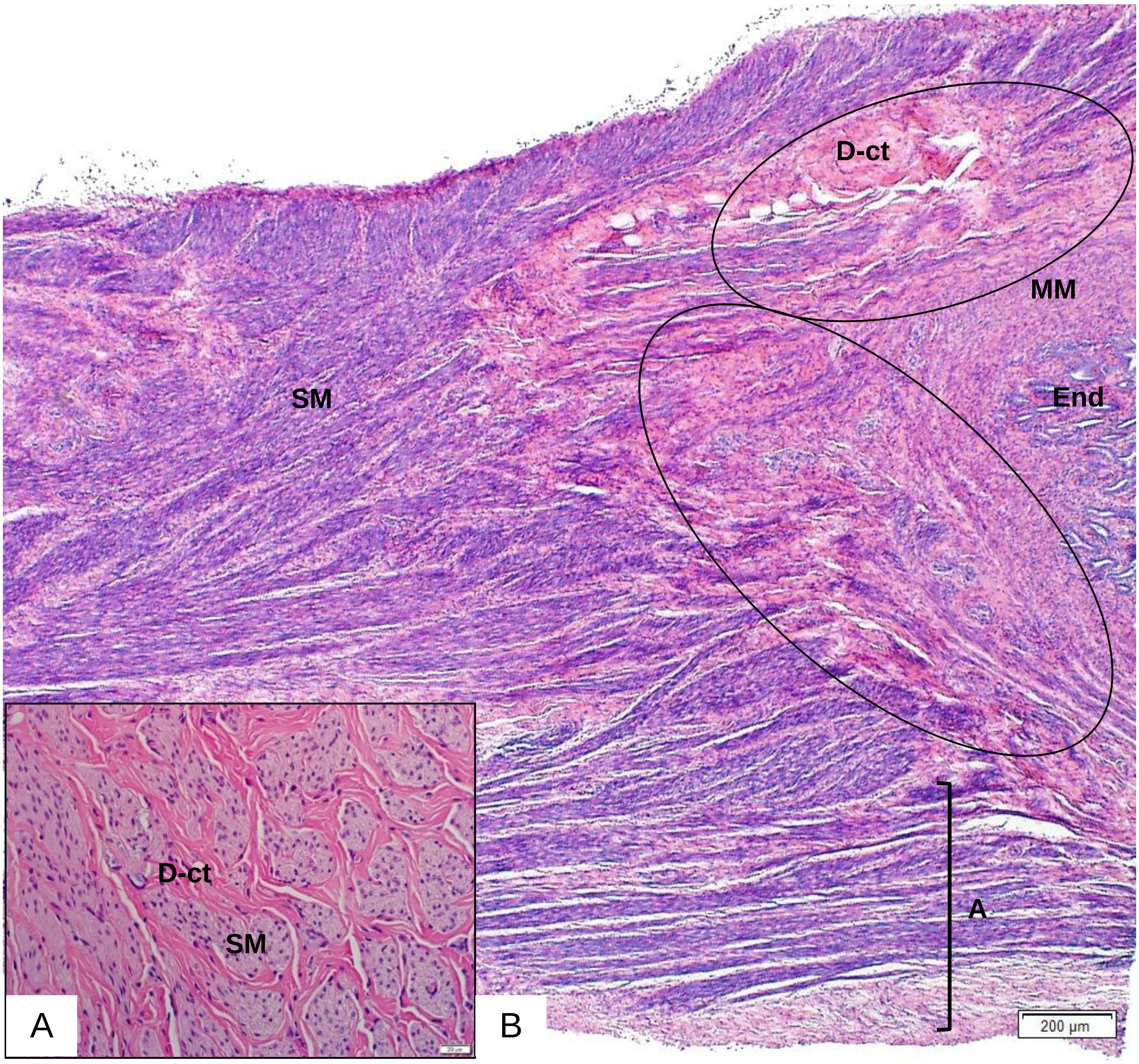


Figure 8.15: A: Transverse section through the proper ligament showing smooth muscle bundles separated by dense, irregular connective tissue (D-ct). Bar = 20 µm

B: Longitudinal section through the proper ligament / uterine horn junction. Some of the proper ligament smooth muscle bundles (SM) terminate on the uterine horn tip by merging with the longitudinal muscle layer of the uterus (black ovals) while the remaining proper ligament smooth muscle bundles continue caudally (A). Myometrium (MM); Endometrium (End). Cranial is left. Bar = 200 µm.

8.2.3. Round ligament

Macroscopically the round ligament terminated on the uterine horn. The uterine sling abutted the medial aspect of the round ligament (Figure 8.16). Histologically the round ligament predominantly consisted of smooth muscle (Figure 8.17) and contained small blood vessels. The smooth muscle of both the round ligament and uterine sling was continuous with the smooth muscle of the broad ligament (Figure 8. 18). At the midsection of the round ligament the smooth muscle was separated into distinct bundles by loose connective tissue and fat cells were occasionally present. At the junction of the round ligament with the uterine horn the smooth muscle of the former terminated on the outer longitudinal smooth muscle layer of the horn. Figure 8.19).

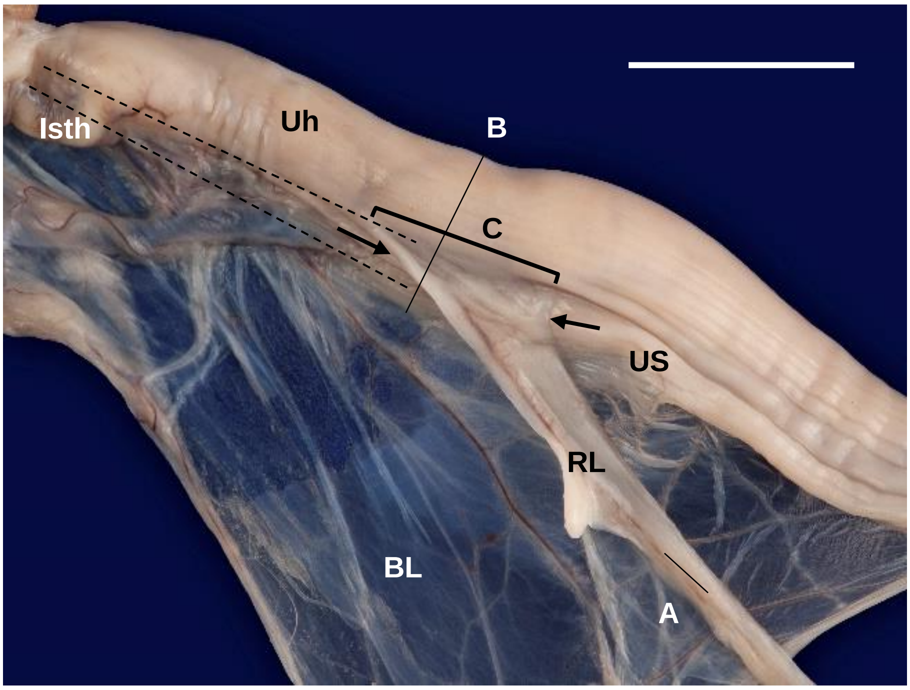


Figure 8.16: Lateral view of the junction of the round ligament (RL) with the left uterine horn (Uh) of a10.5 year-old cheetah. Cranial is left. The round ligament terminates (C) on the uterine horn. The uterine sling (US), a smooth, flat cord running the length of the uterus associated with the mesometrial aspect of the uterine tract, abuts the medial surface of the round ligament (arrows). The uterine sling is hidden cranially by the tip of the uterine horn and broad ligament (BL), and is best viewed from the medial aspect (see Figure 8.3). The black dotted lines indicate the position of the hidden cranial part of the uterine sling. The solid black lines marked A and B indicate the location of the two sections depicted in Figure 8.17: Point A in Figure 8.16: Longitudinal section of the round ligament. Tearing of the tissue is evident (A). Smooth muscle (SM). Bar = 20 µm. & Figure 8. 18. Isthmus (Isth); Bar = 10 mm.

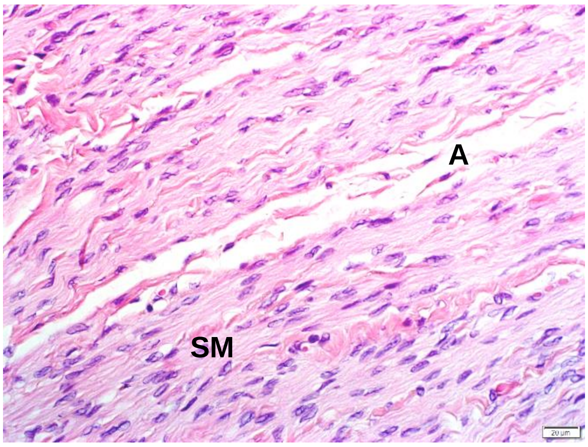


Figure 8.17: Point A in Figure 8.16: Longitudinal section of the round ligament. Tearing of the tissue is evident (A). Smooth muscle (SM). Bar = 20 µm.

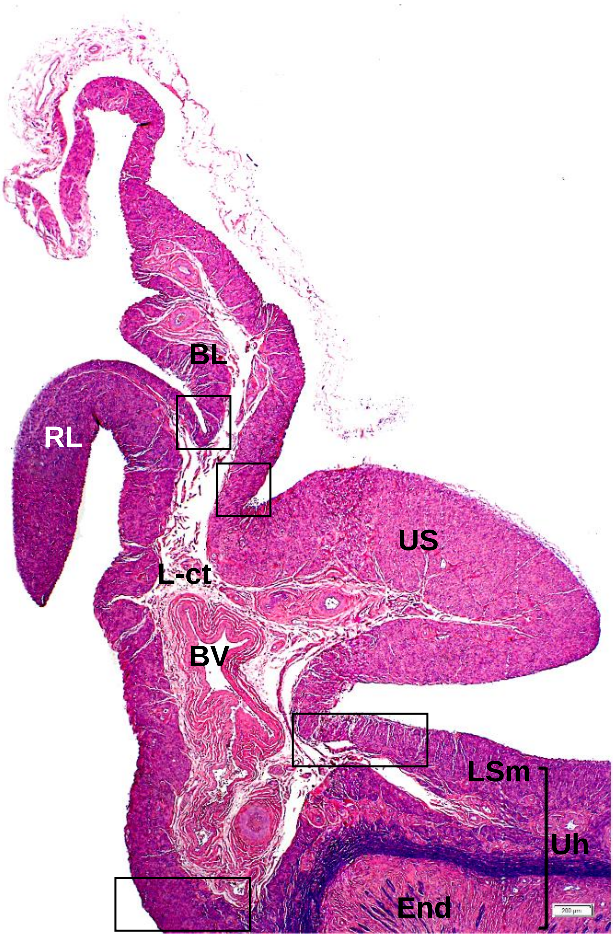


Figure 8. 18: Point B in Figure 8.16: Transverse section near the uterine horn / round ligament junction. The round ligament (RL) and the uterine sling (US) both emanate from thickenings of the smooth muscle that are continuous with the longitudinal smooth muscle of the myometrium (LSm) (black rectangles) and broad ligament (BL) (black squares). Endometrium (End); Blood vessels (BV); Loose connective tissue (L-ct) . Bar = 200 µm.

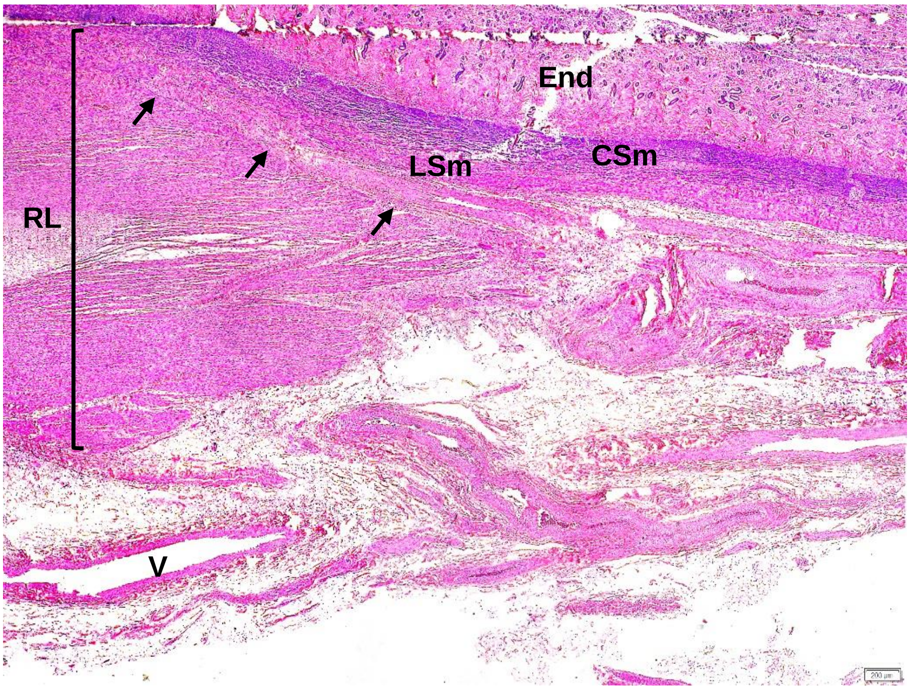


Figure 8.19: Junction of the round ligament (RL) with the uterine horn. Cranial is left. The round ligament smooth muscle fibres terminate on the myometrium (arrows). Endometrium (End); Circular smooth muscle of the myometrium (CSm); Longitudinal smooth muscle of the myometrium (LSm); Veins (V). Bar = 200 µm.

8.2.4. Intercornual ligament

The intercornual ligament was predominantly composed of smooth muscle fibres arranged in two sheets and between which was a layer of loose connective tissue housing a well-developed vascular plexus continuous with that of the *Stratum vasculare* of the uterus (Figure 8.20 A). The smooth muscle was continuous with the outer longitudinal layer of the myometrium (Figure 8.20 A&B). Small amounts of dense connective tissue surrounding the smooth muscle bundles. Small blood vessels were present between the muscle fibres (Figure 8.20 C).

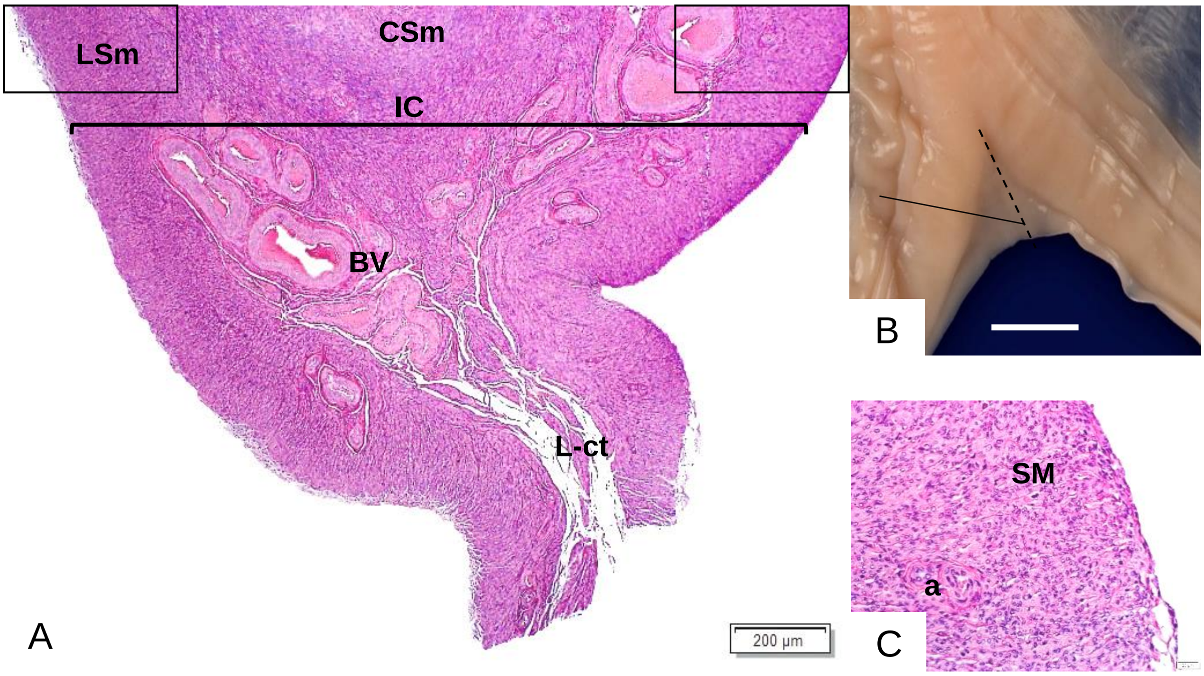


Figure 8.20: A: The intercornual ligament (IC) consists predominantly of smooth muscle with some loose connective tissue (L-ct). The outer longitudinal smooth muscle of the uterus (LSm) is continuous (black rectangels) with the smooth muscle of the intercornual ligament. Circular smooth muscle of the uterus (CSm); Blood vessels forming a vascular plexus (BV). Bar = 200 µm.

B: Macroscopic view of the intercornual ligament showing how it was sectioned. It is not a very substantial structure. The intercornual ligament was split at its midpoint (broken black line) and sectioned transversely through the uterine horn (solid black line). Bar = 5 mm

C: Transverse section of the smooth muscle (SM) of the intercornual ligament with two arterioles (a) present. Bar = 20 µm.

8.2.5. Broad ligament

The broad ligament, a sheet-like mesentery, suspended the uterus and uterine tube from the dorso-lateral body wall (personal communication Dr L du Plessis). The broad ligament was subdivided into the mesometrium, mesovarium and mesosalpinx.

8.2.6. Mesometrium

At the attachment of the mesometrium to the uterine horns and uterine body there was a conspicuous smooth muscle component, macroscopically visible as the uterine sling, which was continuous with the outer longitudinal muscle layer of the uterine body and horns (Figure 8.21). The uterine sling was composed of two well-developed layers of smooth muscle enclosing a layer of connective tissue housing a vascular plexus, lymphatics and nerves supplying the reproductive tract. The vascular layer was continuous with the *Stratum vasculare* of the uterus. The uterine sling tapered evenly from its attachment to the uterus before macroscopically disappearing (Figure 8.5). At this point the smooth muscle of the uterine sling was continuous with that of the mesometrium. The smooth muscle of the mesometrium was separated into bundles by small amounts of dense connective tissue which became more outspoken the further from the uterus the smooth muscle was located (Figure 8.21 A). Eventually the smooth muscle bundles of the broad ligament became separated by more loosely arranged connective tissue (Figure 8.21 B). Smooth muscle remained the dominant tissue type throughout the mesometrium. The entire broad ligament was covered by peritoneal mesothelium.

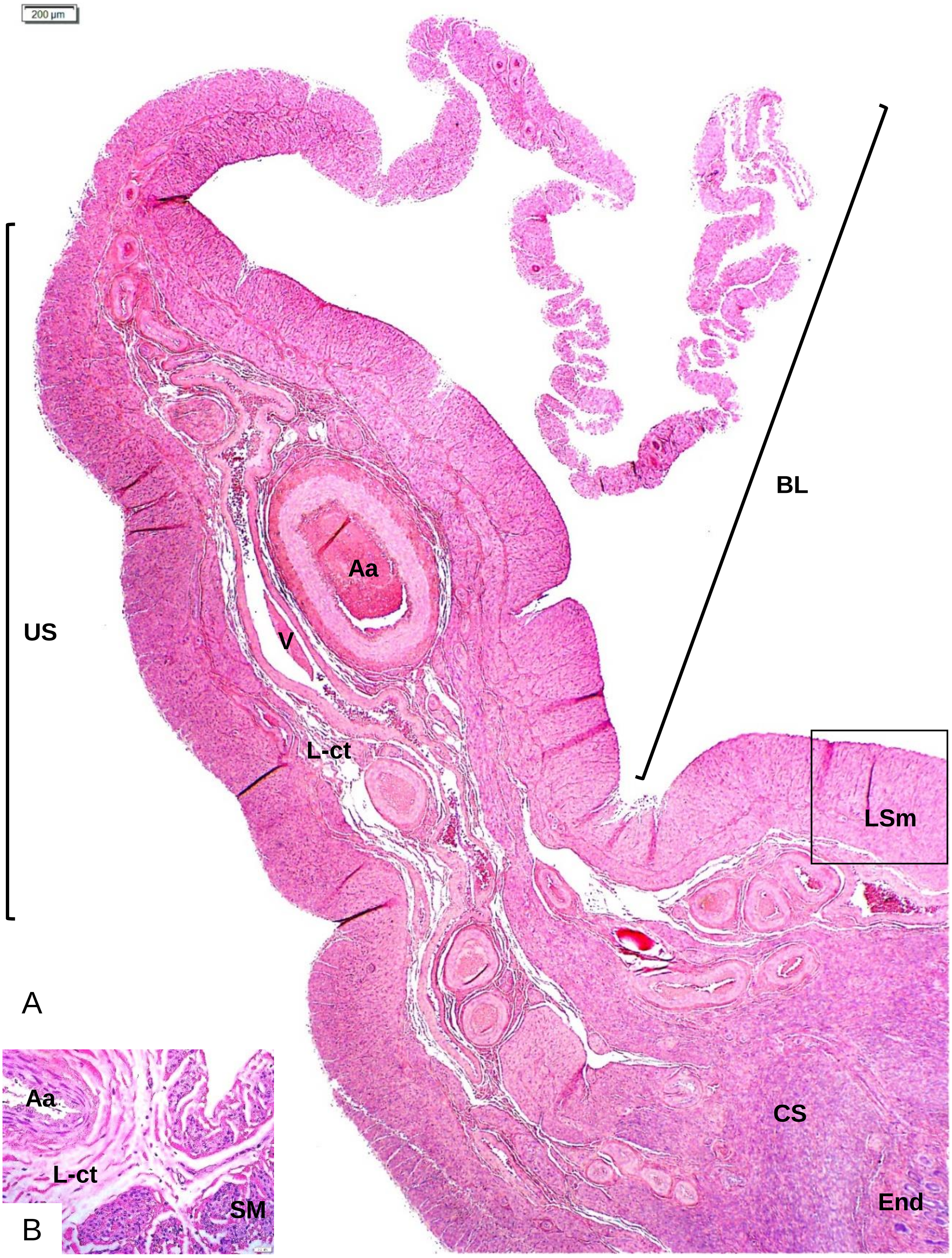


Figure 8.21: A:Transverse section of the broad ligament (BL) at the level of the junction of the uterine horn with the uterine body . The broad ligament adjacent to the uterus is composed primarily of two layers of peripherally arranged smooth muscle enclosing a vascular plexus and connective tissue. The smooth muscle of the broad ligament is continuous with the longitudinal smooth muscle layer of the uterus (black square). Arteries (Aa); Veins (V); Loose connective tissue (L-ct); Longitudinal smooth muscle of the myometrium (LSm); Circular smooth muscle of the myometrium (CSm); Endometrium (End); Smooth muscle band associated with the uterus constituting the uterine sling (US), that tapers into the broad ligament. Bar = 200 µm.

B: Middle of the broad ligament showing an arteriole (aa) surrounded by loosely arranged connective tissue (L-ct) and sandwiched between smooth muscle bundles (SM). Bar = 200 µm.

8.2.7. Mesosalpinx

The mesosalpinx consisted of a layer of peritoneal mesothelium overlying smooth muscle bundles and intervening, loosely arranged connective tissue which contained small blood vessels and nerves. Large blood vessels forming a vascular plexus were sandwiched between the outer longitudinal smooth muscle layer and the inner circular smooth muscle layer of the uterine tube at the attachment to the mesosalpinx (Figure 8.22).

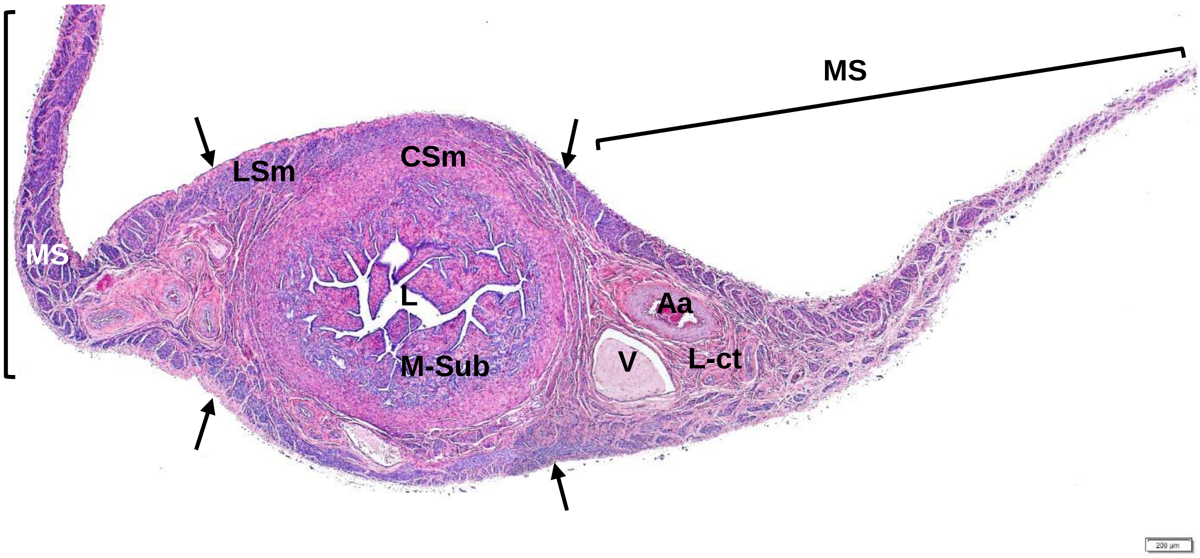


Figure 8.22: Transverse section through the middle section of the ampulla of the uterine tube. The outer longitudinal smooth muscle layer (LSm) of the ampulla is continuous with the prominent longitudinal smooth muscle associated with the mesosalpinx (MS) at its attachments to the uterine tube (arrows). Large blood vessels are sandwiched between the outer longitudinal smooth muscle layer and the inner circular smooth muscle layer of the uterine tube at the junction with the mesosalpinx. Inner circular smooth muscle layer (CSm). Lumen (L); Mucosa-submucosa (M-sub); Veins (V); Arteries (Aa); Loose connective tissue (L-ct). Free margin of the mesosalpinx is to the right. Bar = 200 µm.

8.3. Ovary

The cheetah ovary was ovoid, attached to the free border of the suspensory ligament and consisted of four major histological layers: Germinal epithelium, *Tunica albuginea* (Ovarian capsule), cortex and medulla.

8.3.1. Germinal Epithelium

The germinal epithelium covered the ovarian surface and was continuous with the mesothelium covering the suspensory and proper ligaments. It was comprised of a simple cuboidal epithelium in younger cheetahs as well as in older cheetahs (***Figure 8.23***).

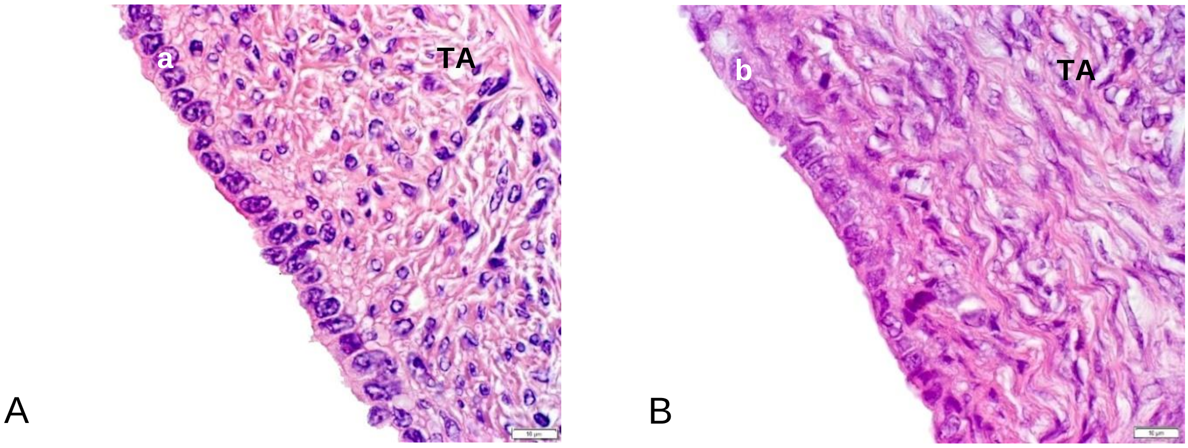


Figure 8.23: A: A simple cuboidal epithelium (a) covers the ovary in a 3 year-old cheetah, as well as in B: an older 10.5 year-old cheetah. *Tunica albuginea* (TA). Bar = 10 µm.

8.3.2. Tunica albuginea

The germinal epithelium was supported by the *Tunica albuginea*, a dense, irregular connective tissue (Figure 8.24) devoid of follicles. Beneath the *T. albuginea* the ovarian cortex began and extended to the medulla.

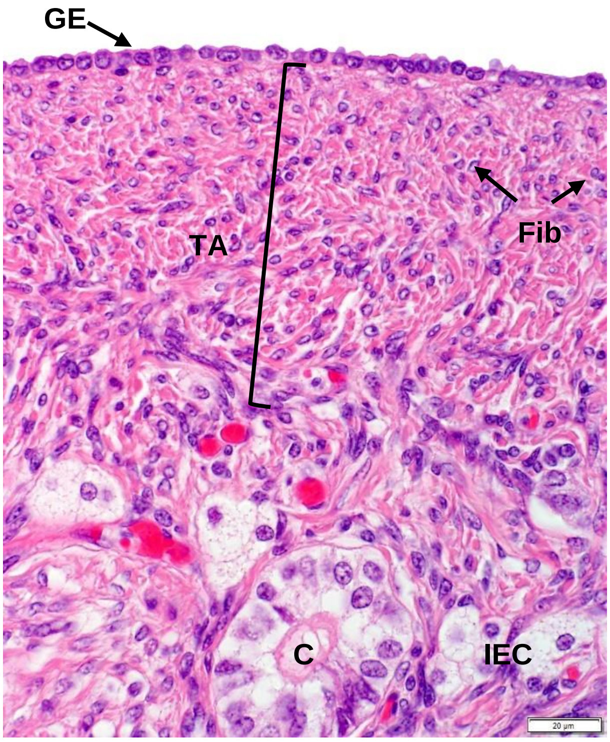


Figure 8.24: Transverse section through the ovary showing the germinal epithelium (GE) and *Tunica albuginea* (TA) of a younger a 3 year-old cheetah composed of dense, irregular connective tissue with fibroblasts (Fib). Primary follicle – Class C (C)*; Interstitial endocrine cells (IEC). Bar = 20 µm.

* The follicle classification system as contemplated in Table 8.4 was used in this section.

8.3.3. The Cortex

The ovarian cortical stroma consisted of or contained the following structures (Figure 8.25): Dense, irregular connective tissue. Spindle shaped fibroblasts were irregularly arranged in the stroma, orderly arranged around follicles and vessels, or orientated parallel to the ovarian surface. Oocytes, consisting of a nucleus and ooplasm aggregated adjacent to the *Tunica albuginea*. Follicles at different stages of development (see folliculogenesis below). Anovular and atretic follicles. Interstitial endocrine cells. Blood and nerve supply. No *Corpora atretica* were present. No *Corpora lutea* were seen.

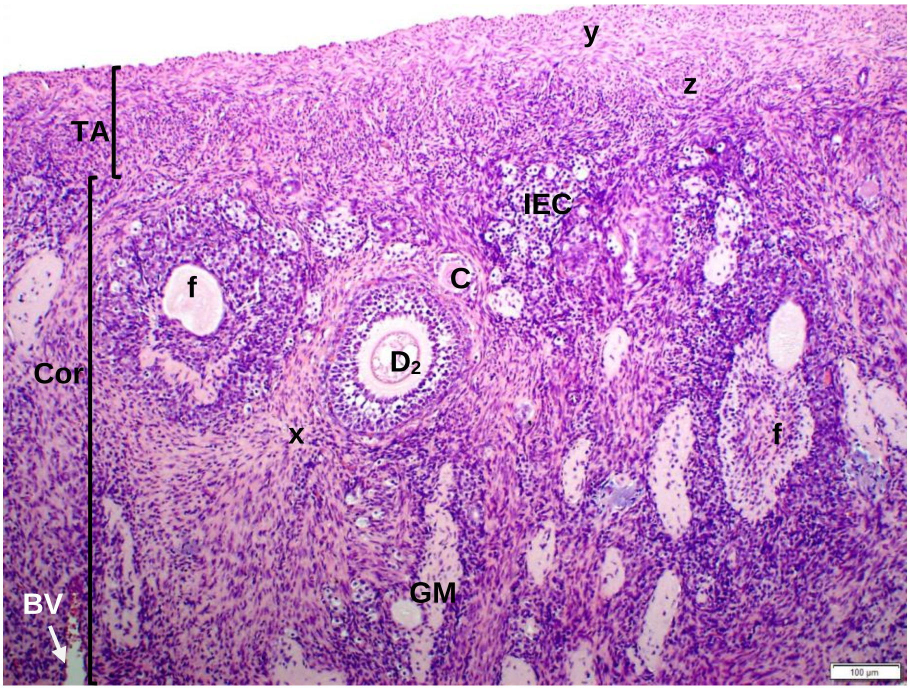


Figure 8.25: Ovarian cortex. Follicles at different stages of development and atresia are present. Fibroblasts form an integral part of the cortex and can be seen orderly arranged (x) around follicles, parallel (y) to the ovarian surface or more irregularly arranged (z) in other places. A blood vessel (BV) is seen infiltrating the cortex in the left of the picture and interstitial endocrine cells (IEC) are present. Multiple glassy membrane remnants (GM) associated with moderate atresia are present. Primary follicles – Class C (C); Early secondary follicle, undergoing atresia (D2). Other follicles in more advanced stages of atresia (f); Tunica albuginea (TA); Cortex (Cor). Bar = 100 µm.

8.3.4. The Interstitial glands (interstitial endocrine cells)

The interstitial endocrine cells formed loose clusters, had dark staining nuclei and a clear cytoplasm. They were commonly observed beneath the *Tunica albuginea* in the vicinity of atretic follicles. The interstitial endocrine cells were often intermingled with the hypertrophied *Theca interna* cells and were similar in appearance albeit larger (Figure 8.26).

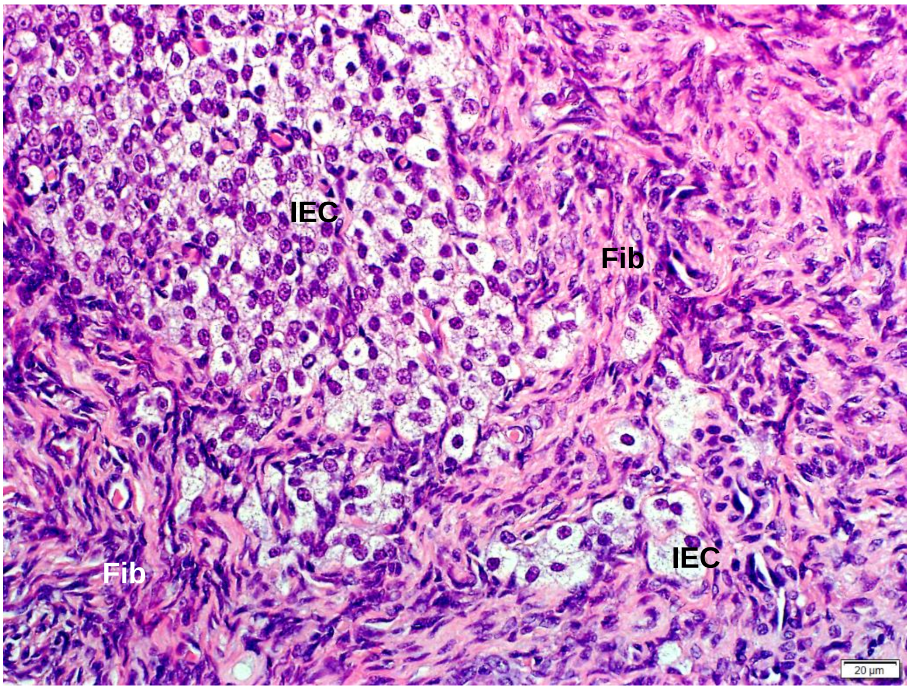


Figure 8.26: Clusters of Interstitial endocrine cells with dark staining nuclei and a clear cytoplasm (IEC) surrounded by fibroblasts (Fib). Bar = 20 µm.

8.3.5. The Medulla

The medulla was highly vascularised, contained irregular dense connective tissue cells and some smooth muscle fibres. Blood supply was via the ovarian artery which entered at the hilus. Branches extended into the cortex to supply the theca of developing follicles and stromal elements. Capillary networks completely surrounded developing follicles (Figure 8.34). Venous drainage mirrored the arterial setup but veins sometimes coalesced to form a venous plexus (Figure 8.27) in the medulla before exiting the hilus. *Rete ovarii* were present and consisted of channels lined by a simple cuboidal epithelium or short, solid cords of epithelial cells. They were found in the hilus region and extended cranially into the suspensory ligament (Figure 8.28; Figure 8.29). Nerve fibres were present in the medulla.

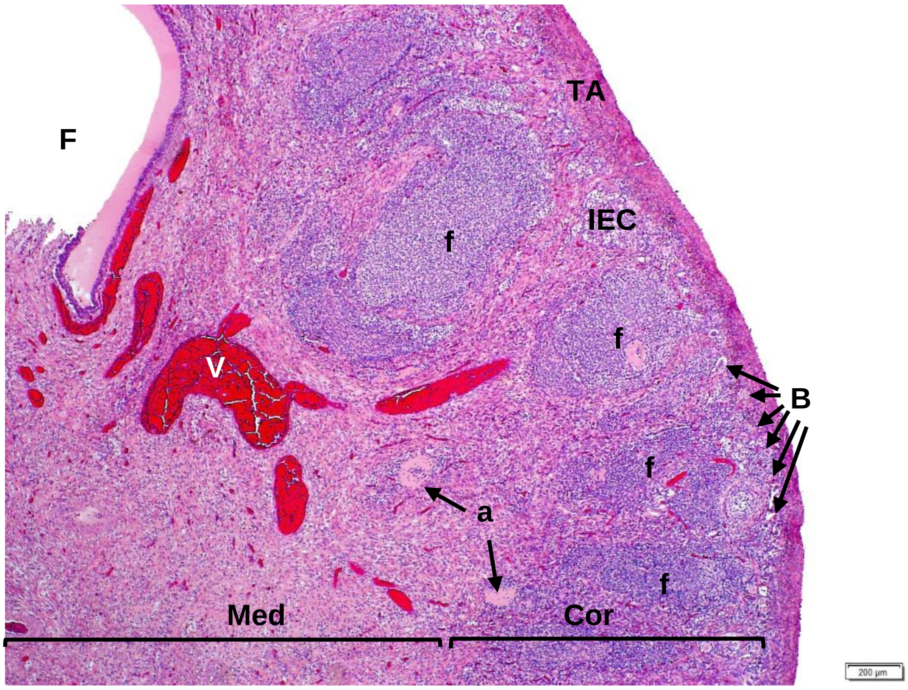


Figure 8.27: Transverse section of the ovary showing an extensive venous plexus (V) in the medulla at the cortico-medullary junction. Multiple follicles at an advanced stage of atresia are present (f) including an atretic tertiary follicle – class F (F). Primordial follicles (B) are concentrated under the *Tunica albuginea* (TA) with many interstitial endocrine cells (IEC); Cortex (Cor); Medulla (Med); Glassy membrane remnants associated with moderate atresia (a). Bar = 200 µm.

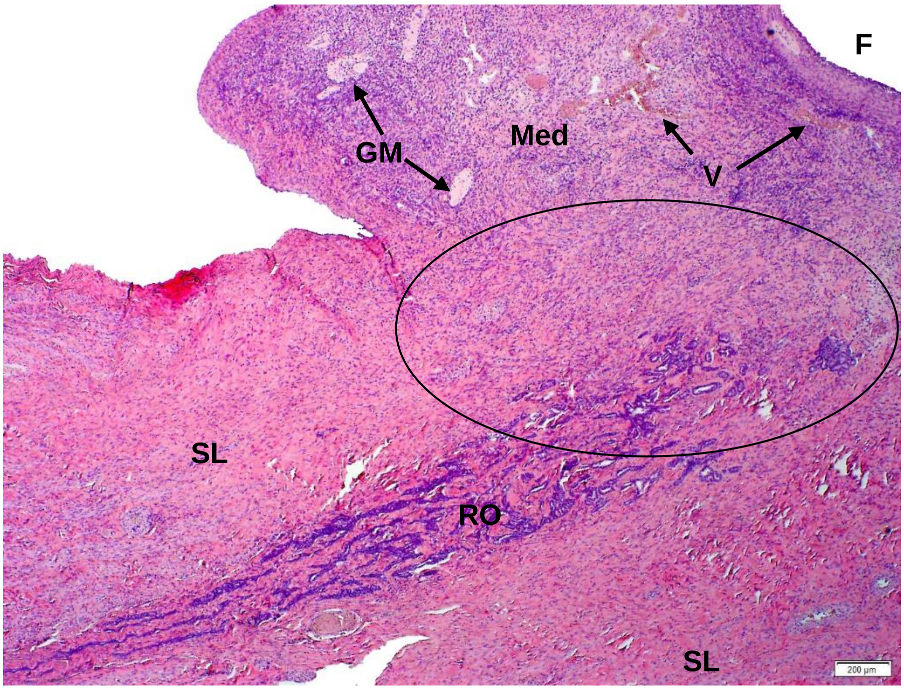


Figure 8.28: Longitudinal section through the tubal extremity of the ovary and the suspensory ligament (SL). Cranial is left. *Rete ovarii* (RO) are present in the hilus in this region and extend cranially into the suspensory ligament. A tertiary follicle – Class F (F) is present in the top right corner. Glassy membrane remnants (GM); Medulla (Med); Veins (V); Hilus (black oval). Bar = 200 µm.

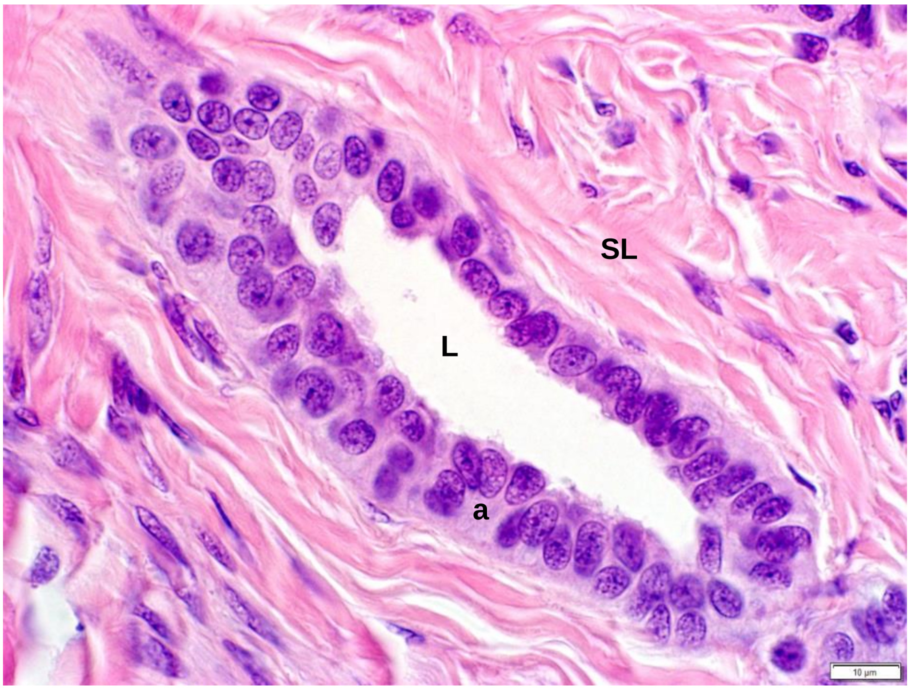


Figure 8.29: Close up of the *Rete ovarii*. A simple cuboidal epithelium (a) lines the lumen (L) of the channels. Irregularly arranged connective tissue of the suspensory ligament (SL). Bar = 10 µm.

8.3.6. Folliculogenesis

Different stages of folliculogenesis were observed. Oogonia were not present. Follicle classes B-F (Primordial to Tertiary follicle) were observed (Table 8.4). Follicle class G and H (Graafian and Pre-ovulatory follicles) and *Corpora lutea* were not observed. Multi-oocyte follicles were not observed, and no scar tissue from atretic follicles was present.

The primordial (class B) follicles (Figure 8.30) were concentrated under the *Tunica albuginea*. Cohorts of primordial follicles were recruited and developed during the reproductive cycle while others remained quiescent. There was no particular spatial pattern of selection of these primordial follicles. Early primary (class B/C) (Figure 8.30) and primary (class C) follicles (Figure 8.31) appeared to be randomly interspersed among the primordial follicles.

A predictable pattern of development from primordial (class B) follicle to tertiary follicle was observed as follows: Primordial (class B) follicles consisting of a primary oocyte surrounded by a single layer of follicular cells started to grow, presumably under the influence of follicle stimulating hormone. As soon as the follicular cells become mitotically active they become known as granulosa cells [44]. The granulosa cells multiplied and differentiated to form different histological structures and layers around the oocyte thereby enlarging the developing follicle. The surrounding stromal elements and fibroblasts formed the follicular theca (Figure 8.32). The granulosa cells immediately surrounding the ovum became columnar in nature and formed the *Corona radiata* with its associated thick, PAS-positive, amorphous basement membrane called the *Zona pellucida* (Figure 8.32). The other granulosa cell layers progressed from squamous to cuboidal to stratified forming the *Membrana granulosa* surrounded by a basement membrane which separated it from the *Theca interna*. An extensive vascular network was present between the basement membrane and the *Theca interna* as well as surrounding the *Theca externa* of the follicular theca (Figure 8.34 & Figure 8.35). The stratified *Stratum granulosum* then started to accumulate *Liquor folliculi* extracellularly in clefts (Figure 8.33) which ultimately coalesced to form one large cavity, the antrum, which surrounded the ovum (Figure 8.34; Figure 8.35; Figure 8.36). The follicular theca continued to differentiate, the fibroblasts changed into epithelioid cells in the *Theca interna* and remained as a dense coat of fibroblasts in the *Theca externa* (Figure 8.34). The granulosa cells were divided by the developing antrum into a parietal layer, which did not directly surround the developing ovum, called the *Stratum granulosum*. The ovum was located to one side of the fluid filled antrum embedded in a mound of granulosa cells, known as the *Cumulus oophorus* or *Discus proligerus*, which constituted the visceral portion of the granulosa cells (Figure 8.36). At this point the follicle had reached the tertiary follicle (class F) stage. The follicle was growing down into the depths of the cortex displacing cortical elements out of its way as it expanded. No Graafian or pre-ovulatory follicles were documented.

Table 8.4: Follicle morphological classification used in this dissertation. * Only morphological criteria from Lintern-Moore et. al. have been used (class A-F). Oocyte and follicle size, commencement of oocyte growth and follicle cell numbers have been omitted. The final two thesis terms (blue shading), their associated suggested class and criteria have been chosen using observation in the literature of a distinctive stalk [50,57] and a free floating ovum prior to ovulation [49,50].

Dissertation terms	Lintern-Moore et al. Class [56]	Criteria *
Primary oocyte	Class A oocyte	Primary oocyte with no associated granulosa cells.
Primordial follicle	Class B follicle	Primary oocyte with one layer of squamous granulosa cells (Figure 8.30).
Early primary follicle	Class B/C follicle	Primary oocyte with one layer of squamous & cuboidal granulosa cells (Figure 8.30).
Primary follicle	Class C follicle	Primary oocyte with one layer of cuboidal granulosa cells and a complete basement membrane separating them from a halo of connective tissue fibres and fibroblast-like cells with no definitive theca present. (Figure 8.31).
Late primary follicle	Class D1 follicle	Oocyte with 2-7 layers of granulosa cells, a clearly differentiated thecal layer, and a *Zona pellucida* (Figure 8.32).
Secondary follicle	Class D2 follicle	Call-exner vacuoles present in the granulosa cells and or fluid accumulation between the granulosa cells (Figure 8.33).
Early Tertiary follicle	Class E follicle	Crescent shaped antrum (Figure 8.34; Figure 8.35).
Tertiary follicle	Class F follicle	*Cumulus oophorus* is present and thecal cells may be hypertrophied. Advanced vascularisation of the theca is present (*Figure 8.36).*
Graafian follicle	Class G follicle	*Cumulus oophorus* has developed a distinctive stalk [50,57].
Pre-ovulatory follicle	Class H follicle	Ovum is floating free in the antrum. Antral wall bulges from the ovary surface [49,50].

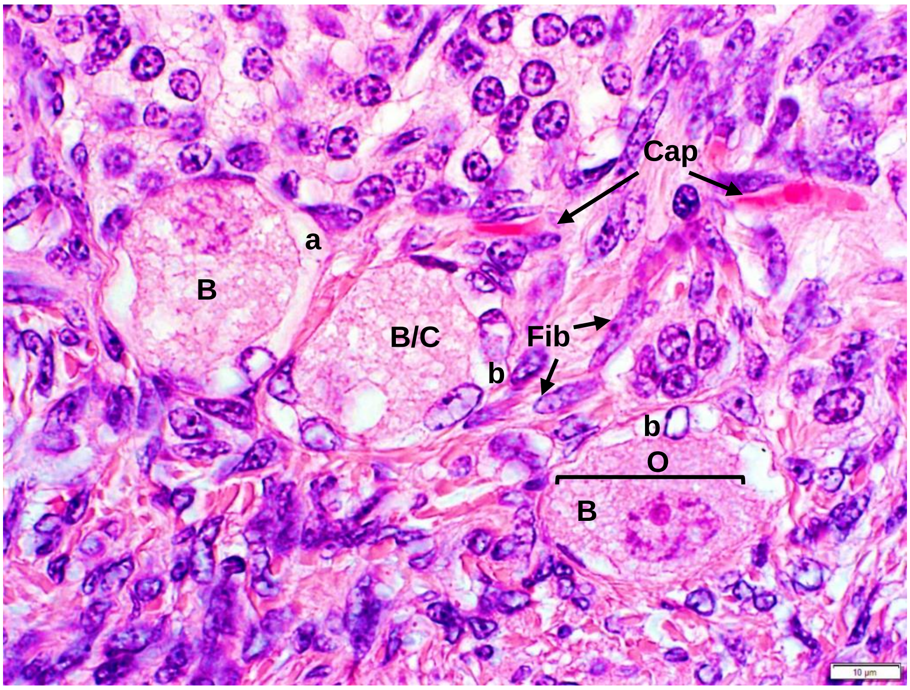


Figure 8.30: Primordial - Class B follicles (B) consisting of a primary oocyte (O) surrounded by a single layer of squamous epithelium (a). Early primary – Class B/C follicles (B/C) consisting of a primary oocyte with a single layer of squamous and cuboidal epithelium (b). Fibroblasts (Fib); Capillaries (Cap). Bar = 100 µm.

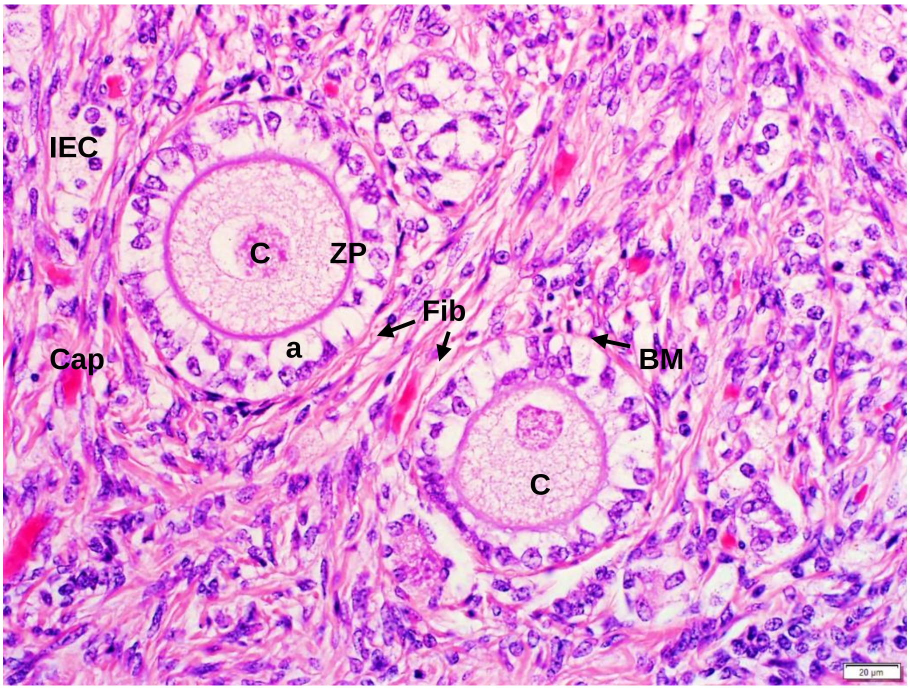


Figure 8.31: Primary – Class C follicles (C). The primary oocyte is surrounded by a single layer of cuboidal epithelium (a) resting on a complete basement membrane (BM) and surrounded by a halo of fibroblasts (Fib) and connective tissue fibres. Interstitial endocrine cells are present (IEC) and capillaries (Cap). The *zona pellucida* is just starting to form (ZP). Bar = 20 µm.

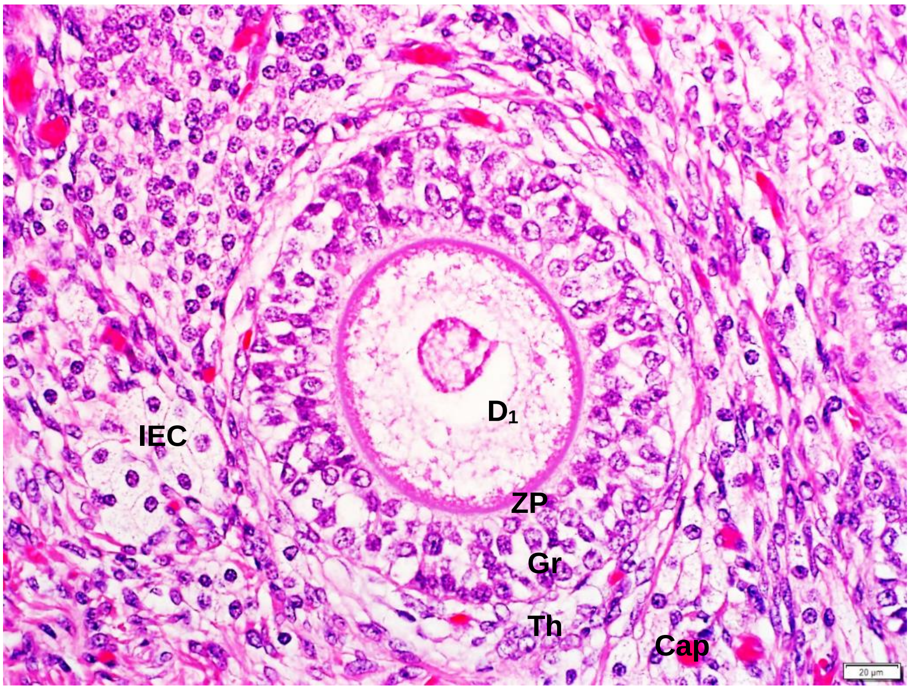


Figure 8.32: Late primary – Class D1 follicle (D1). The oocyte is surrounded by a *Zona pellucida* (ZP), 2-7 layers of granulosa cells (Gr), and a clearly differentiated thecal layer (Th). Interstitial endocrine cells (IEC) and capillaries are present (Cap). Bar = 20 µm.

Figure 8.33: Secondary – Class D2 follicle. Fluid accumulation is starting (a) between the granulosa cells (Gr). Nucleolus (N); Oocyte (O); *Zona pellucida* (ZP); *Corona radiata* (CR); Theca (Th); Capillaries (Cap). Bar = 20 µm.

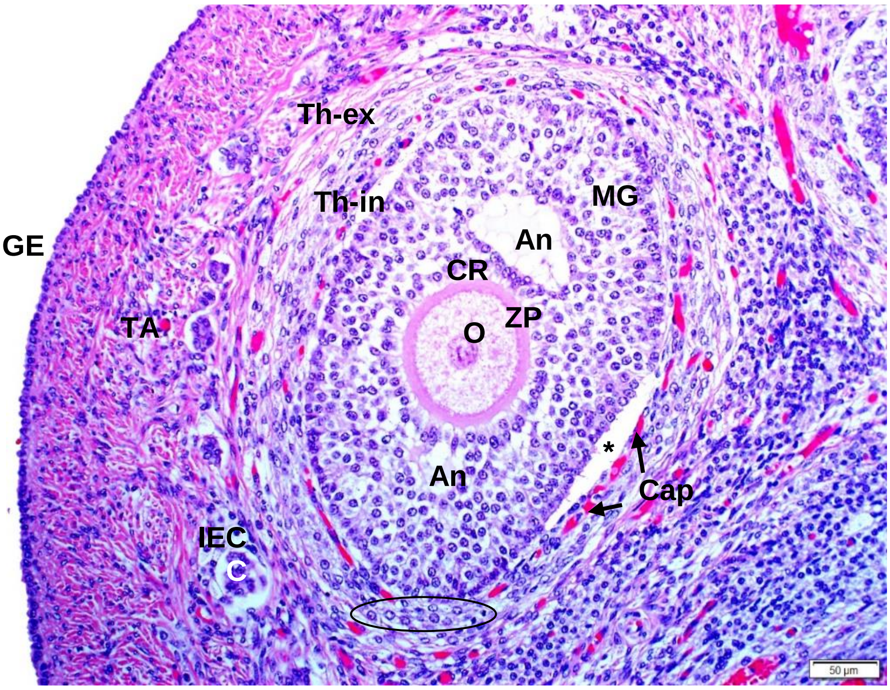


Figure 8.34: Early tertiary – Class E follicle. A clear antrum has started forming (An); The fibroblasts of the *Theca interna* (Th-in) have started differentiating into epithelioid cells (black oval). The rich vascular network surrounding the *Membrana granulosa* (MG) and *Theca interna* is clearly visible. Separation of the tissues represents an artefact. Oocyte (O); *Zona pellucida* (ZP); *Corona radiata* (CR); *Membrana granulosa* (MG); *Theca externa* (Th-ex); Capillaries (Cap); *Tunica albuginea* (TA); Germinal epithelium (GE); Interstitial endocrine cells (IEC); Primary – class C follicle (C). Bar = 50 µm.

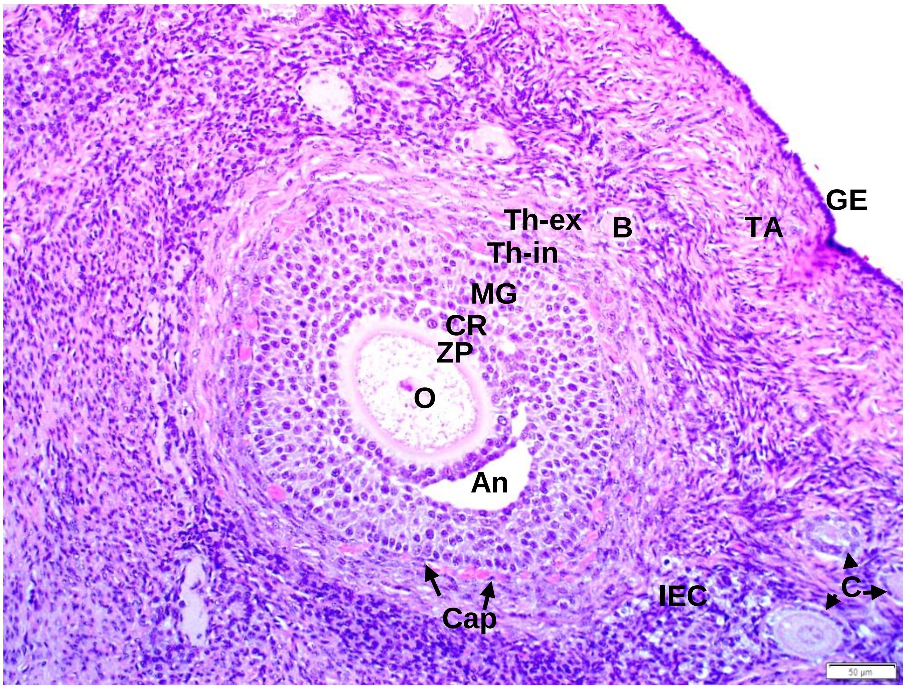


Figure 8.35: Early tertiary – Class E follicle. A clear antrum has started forming (An). The *Theca interna* (Th-in) has started differentiating. The capillary (Cap) network surrounding the *Membrana granulosa* (MG) is evident. Oocyte (O); *Zona pellucida* (ZP); *Corona radiata* (CR); *Theca externa* (Th-ex); Primordial follicles (B); *Tunica albuginea* (TA); Germinal epithelium (GE); Interstitial endocrine cells (IEC); Primary – class C follicles (C). Bar = 50 µm.

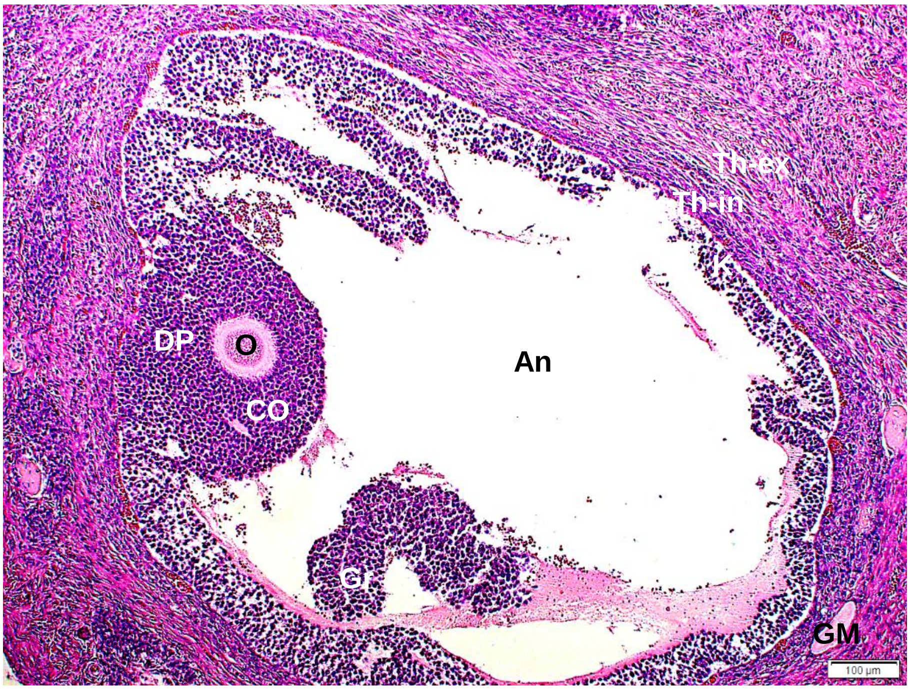


Figure 8.36: Tertiary – Class F follicle. Signs of early atresia are present evidenced by some cell sloughing in the antrum (An), pyknotic granulosa cell nuclei and a swollen *Zona pellucida*, although damage to the granulosa cell layer (Gr) has occurred during processing. The oocyte (O) is located in the *Cumulus oophorus* (CO). Hypertrophied thecal cells are present in the *Theca interna* (Th-in). *Theca externa* (Th-ex); *Discus proligerus* (DP); Glassy membrane remnants (GM). Bar = 100 µm.

8.3.7. Atresia

Anovulatory cheetah follicles underwent obliterative atresia. The basement membrane of early follicle classes did not hypertrophy to form a glassy membrane. The glassy membrane seemed to only be associated with atresia of larger follicle classes. When hypertrophy of the glassy membrane did occur, it did not appear to hypertrophy as much as in humans. Folding of the glassy membrane was observed. All observed classes of follicles (B-F) were seen at various stages of atresia. No scarring was evident from atresia of any follicle classes. The extensive vascular network seen just beneath the basement membrane, and also seen surrounding the *Theca interna,* remained prominent throughout atresia, only regressing in moderate to late atresia (Figure 8.37). The blood vessels surrounding the basement membrane presumably played an important role in supporting the formation and resorption of the glassy membrane.

Atresia advanced as follows in cheetahs.

8.3.7.1. Very early atretic changes

- *Theca interna* and *Membrana granulosa* were intact.
- The antrum still contained follicular fluid.
- Disruption of the *Cumulus oophorus* occurred and degeneration of the ovum advanced.
- A swollen *Zona pellucida* was present (Figure 8.38: Very early atresia of a late primary - Class D1 follicle. The ovum (O) is degenerating and the *Zona pellucida* (ZP) is swollen. The *Membrana granulosa* (MG) and *Theca interna* (Th-in) is intact. Interstitial endocrine cells (IEC); Primary – Class C follicle (C); Fibroblasts (Fib); *Theca externa* (Th-ex); Capillaries (Cap).).

8.3.7.2. Early atresia

- The granulosa cells closest to the antrum hypertrophied and became pyknotic, presumably playing a role in resorbing the follicular fluid, and resulting in a collapse of the antral cavity (Figure 8.39).
- The basement membrane had not started to hypertrophy yet.

8.3.7.3. Advanced early atresia

- The cells of the *Membrana granulosa* continued to resorb follicular fluid until the antrum was completely filled by them (Figure 8.40).
- Pyknosis of the granulosa cells then occurred (Figure 8.40; Figure 8.41).
- The basement membrane thickened in larger follicles (Class D_1 and bigger) to form the glassy membrane and reached its most hypertrophied state (Figure 8.41; Figure 8.42). No glassy membrane formed in smaller atretic follicles.
- Loose connective tissue and capillaries started to grow in from the stroma and *theca externa* to start infiltrating the granulosa cells that filled the antral cavity (Figure 8.43).
- The glassy membrane folded together with hypertrophy (Figure 8.42; Figure 8.43) and was resorbed in places (Figure 8.45). Fibroblasts were seen crossing the glassy membrane together with capillaries (Figure 8.44).

8.3.7.4. Moderate atresia

- The glassy membrane regressed and disappeared completely in most cases. Remnants of hypertrophied glassy membrane remained for some time as the only indication of former atresia and were eventually completely resorbed leaving no scar tissue (*Corpora atretica*). These remnants occurred mainly deeper in the cortex suggesting they may only sometimes form from large tertiary or Graafian follicles. (Figure 8.48)
- Loose connective tissue with small blood vessels completely filled the former antrum progressively replacing the granulosa cells (Figure 8.46; Figure 8.47; Figure 8.48).

8.3.7.5. Late atresia

- Loose connective tissue was replaced by stroma (Figure 8.49; Figure 8.50).

8.3.7.6. Atypical cases

- Occasionally, the glassy membrane was not invaded by fibroblasts nor resorbed. Instead, it showed marked hypertrophy and folding with few granulosa cells remaining. Only one instance, out of the 7 cheetahs and 70 ovarian sections examined, was found in a 10 year-old cheetah, (Figure 8.51).

Figure 8.37: Transverse section of the ovary showing all stages of follicular atresia. Early tertiary – class E follicle (E) showing very early atretic change. Early atresia of a tertiary follicle (a); Advanced early atresia (b); Moderate atresia (c); Late atresia (d). No *Corpora atretica* are present. There is an extensive blood supply to developing (x) and atretic follicles. Vein (V). The *Membrana granulosa* has pulled away from the *theca-interna* of the tertiary follicle and is an artefact. Hilus (black oval); Suspensory ligament (SL). Bar = 200 µm.

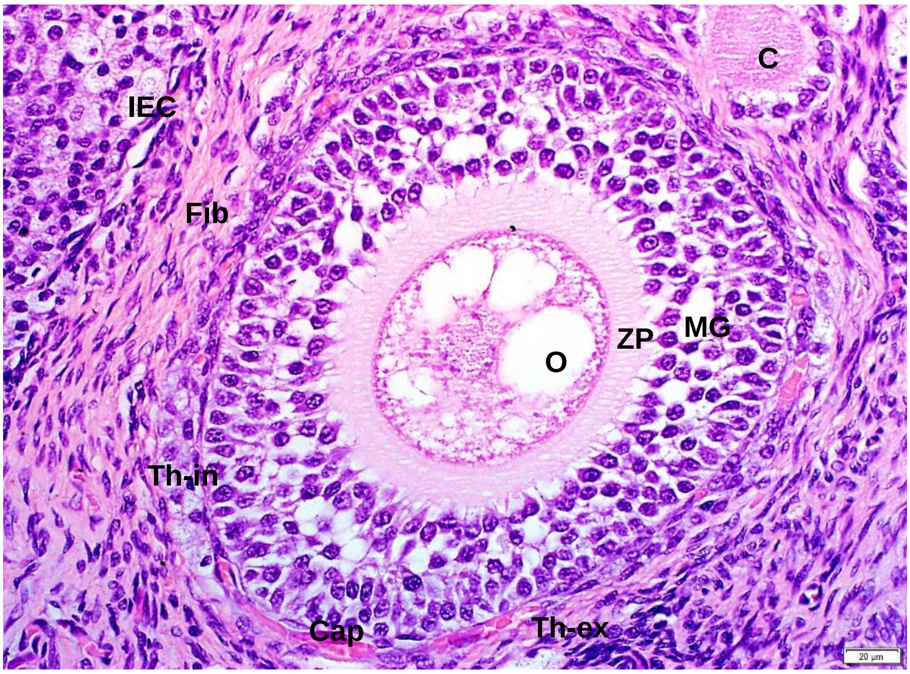


Figure 8.38: Very early atresia of a late primary - Class D1 follicle. The ovum (O) is degenerating and the *Zona pellucida* (ZP) is swollen. The *Membrana granulosa* (MG) and *Theca interna* (Th-in) is intact. Interstitial endocrine cells (IEC); Primary – Class C follicle (C); Fibroblasts (Fib); *Theca externa* (Th-ex); Capillaries (Cap). Bar = 20 µm.

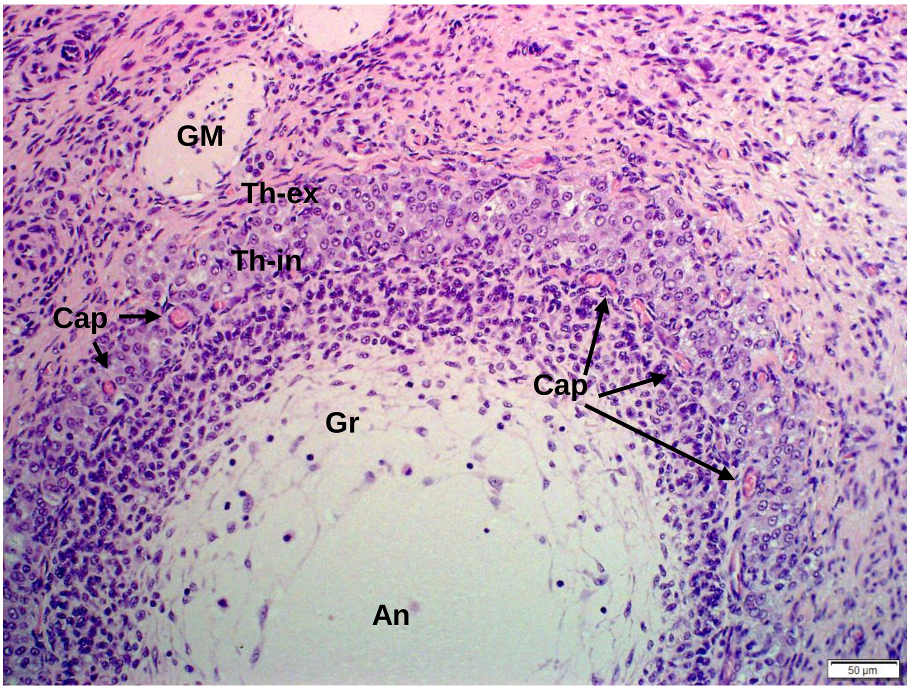


Figure 8.39: Early atresia of a Secondary – Class D2 follicle. The granulosa cells (Gr) are hypertrophying and obliterating the antrum (An). *Theca interna* (Th-in); *Theca externa* (Th-ex); The glassy membrane (GM) remnants of an adjacent atretic follicle in moderate atresia is present. Capillaries (Cap). Bar = 50 µm.

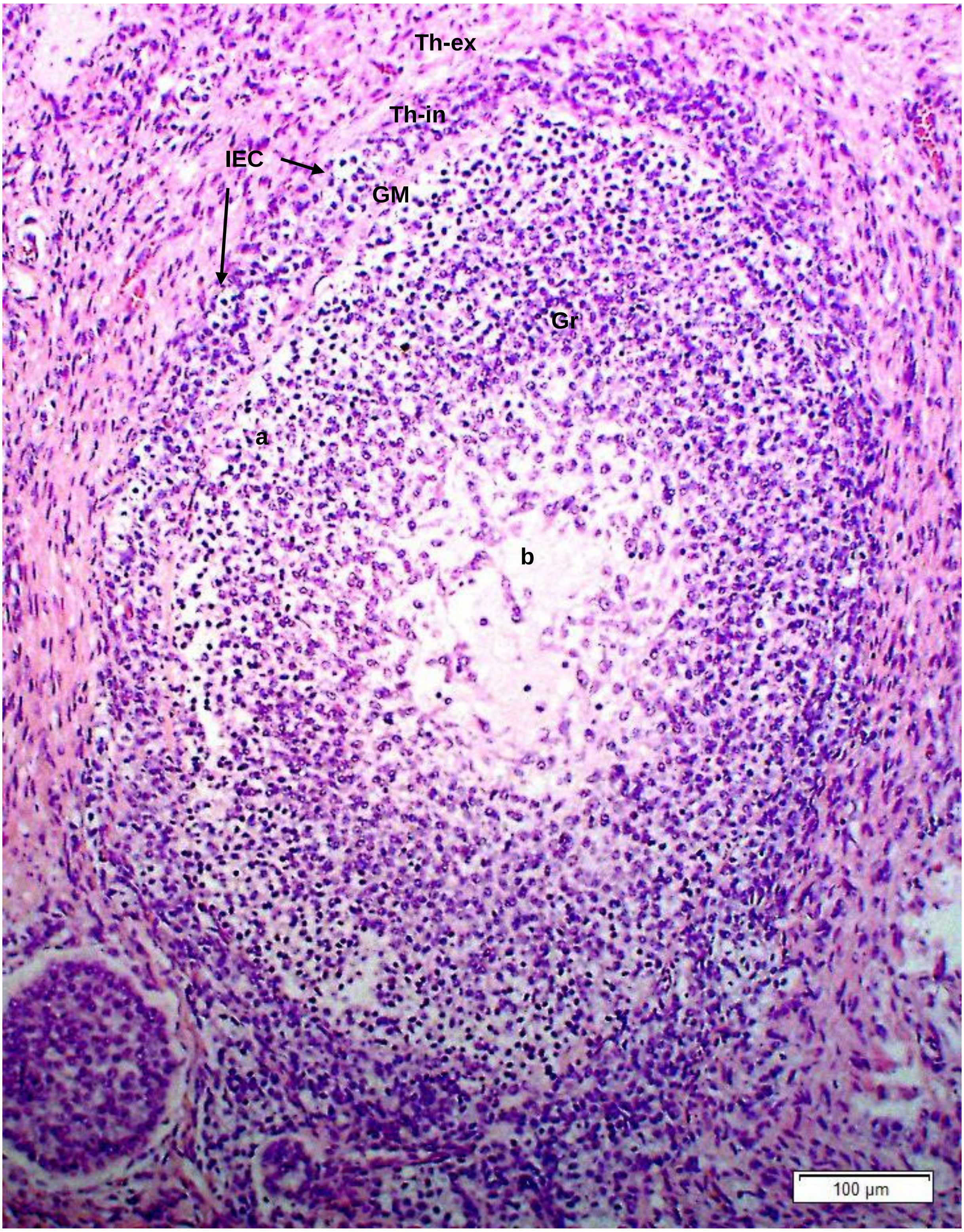


Figure 8.40: Advanced early atresia. The granulosa cells (Gr) have just filled the former antrum. They are starting to undergo pyknosis (a) and be resorbed. The Glassy membrane (GM) is just appearing and follicular fluid has almost been resorbed. Interstitial endocrine cells (IEC) are present scattered among the voluminous *Theca interna cells* (Th-in). A small atretic follicle is visible in the bottom left corner. Hypertrophied granulosa cells (b); *Theca externa* (Th-ex). Scale is 100 µm.

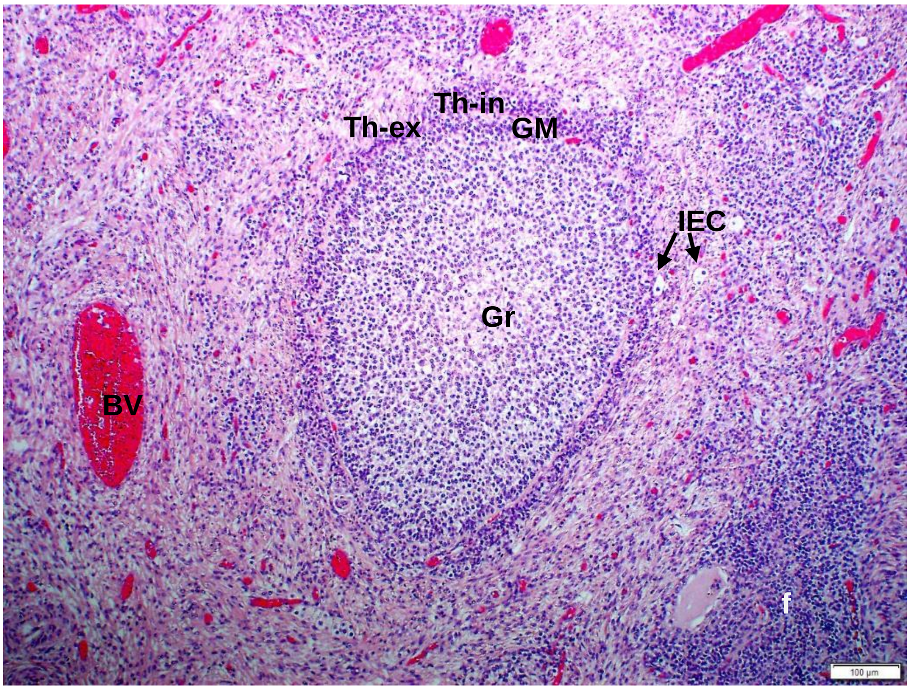


Figure 8.41: Advanced early atresia. The granulosa cells (Gr) have completely filled the former antrum and are noticeably pyknotic. The glassy membrane (GM) is thicker. *Theca externa* (Th-ex); *Theca interna* (Th-in); Interstitial endocrine cells (IEC); Blood vessels (BV). A follicle in moderate atresia is visible with a hypertrophied remnant of glassy membrane in the bottom right corner (f). Bar = 100 µm.

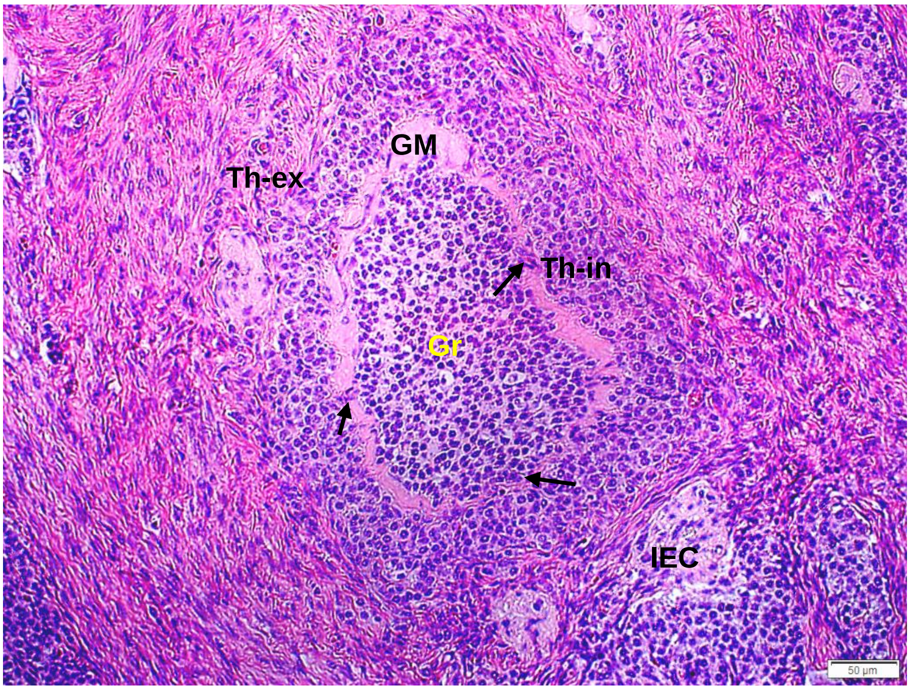


Figure 8.42: Advanced early atresia. The granulosa cells (Gr) have completely filled the former antrum and are pyknotic. The glassy membrane (GM) is noticeably thicker and has started folding. The glassy membrane is being resorbed in places and fibroblasts (arrows) are infiltrating the antrum. The *Theca interna* cells (Th-in) are more voluminous and not pyknotic. The *Theca externa* (Th-ex) is not distinguishable from the surrounding connective tissue. Interstitial endocrine cells (IEC). Bar = 50 µm.

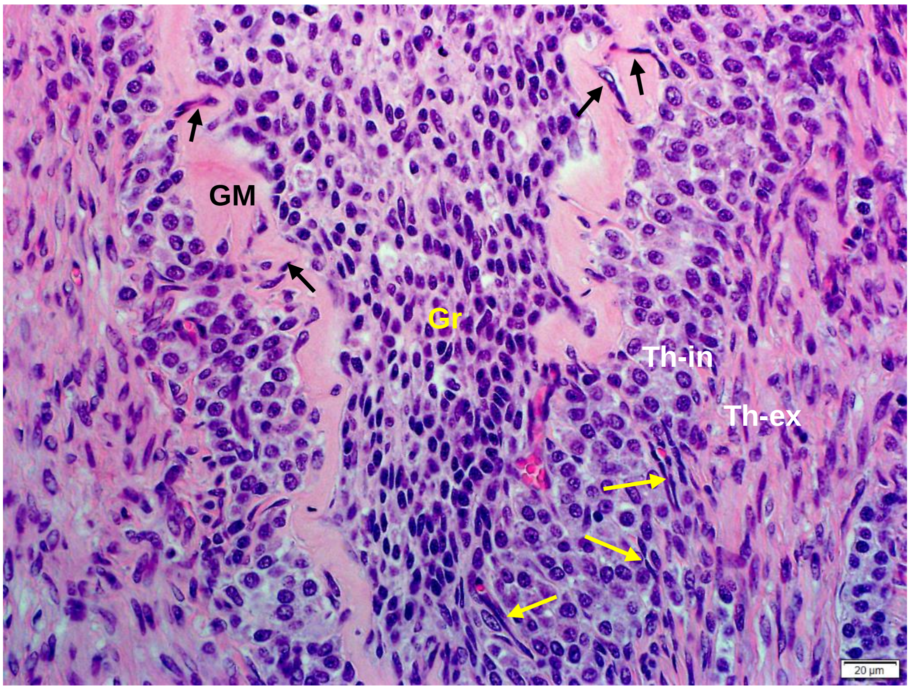


Figure 8.43: Advanced early atresia. Fibroblasts (black & yellow arrows) from the *Theca externa* (Th-ex) are infiltrating between *the Theca interna* cells (Th-in) and crossing the glassy membrane (GM) in places. The glassy membrane is folding and has been partially resorbed. A capillary can be seen running in the area that the glassy membrane used to occupy (red structure). The granulosa cells are noticeably pyknotic (Gr). Bar = 20 µm.

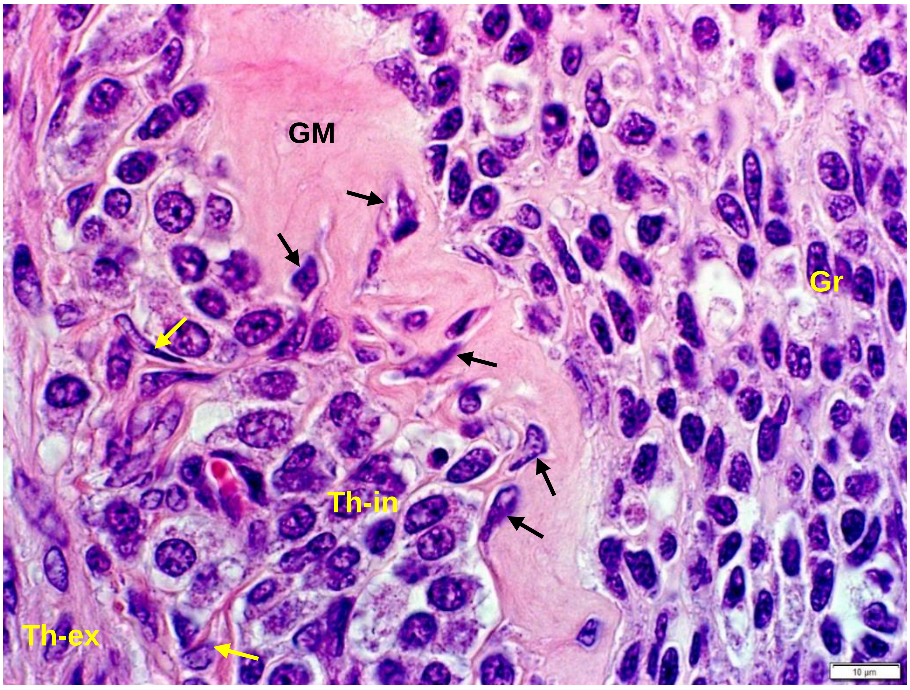


Figure 8.44: Advanced early atresia. Fibroblasts (black and yellow arrows) are seen moving from the *Theca externa* (Th-ex) between the *Theca interna* cells (Th-in) to cross the glassy membrane (GM). The former antrum is filled with granulosa cells (Gr) which are predominantly pyknotic. Bar = 10 µm.

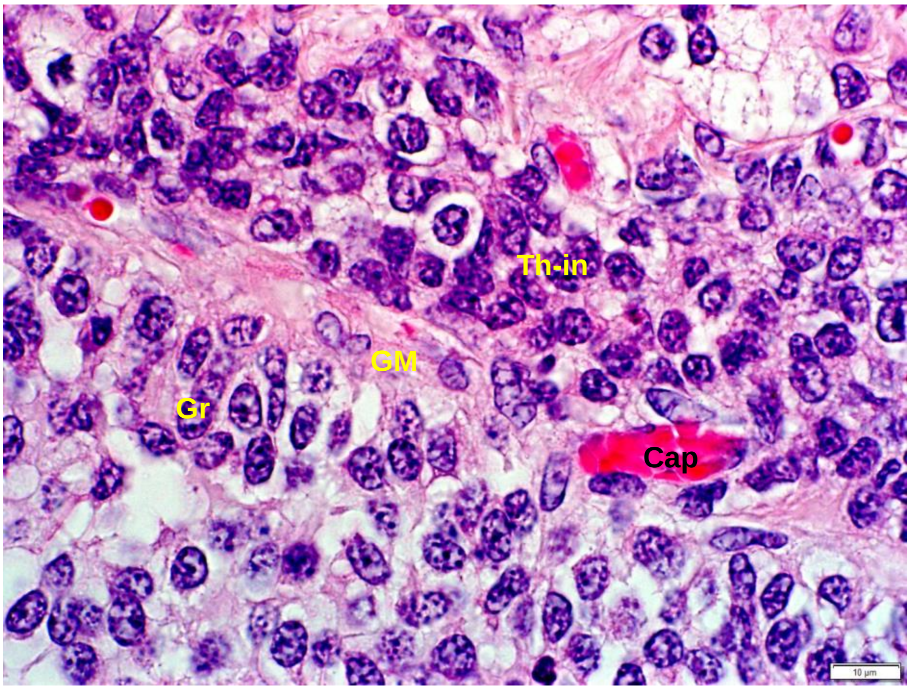


Figure 8.45: Advanced early atresia showing an area of the glassy membrane that has been resorbed. Capillaries (Cap) run parallel to the former glassy membrane (GM) and represent the capillary network enveloping the basal membrane. *Theca interna* (Th-in); Granulosa cells (Gr). Bar = 10 µm.

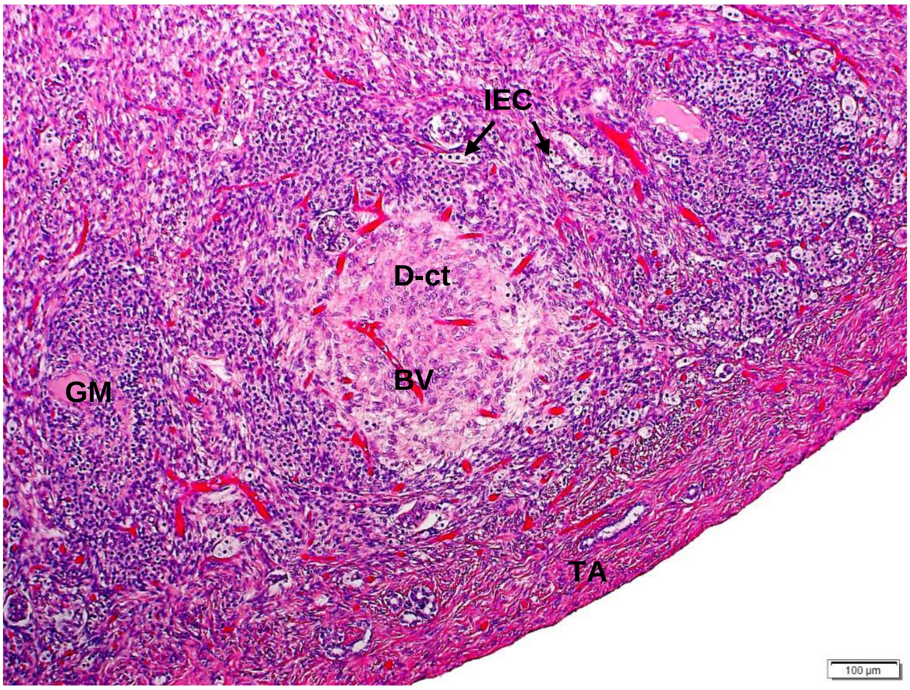


Figure 8.46: Moderate atresia. Dense connective tissue (D-ct) with small blood vessels (BV) completely fill the former follicle replacing the granulosa cells. A glassy membrane does not form in smaller atretic follicles like this one. Tunica albuginea (TA); Glassy membrane remnants (GM); Interstitial endocrine cells (IEC). Bar = 100 µm.

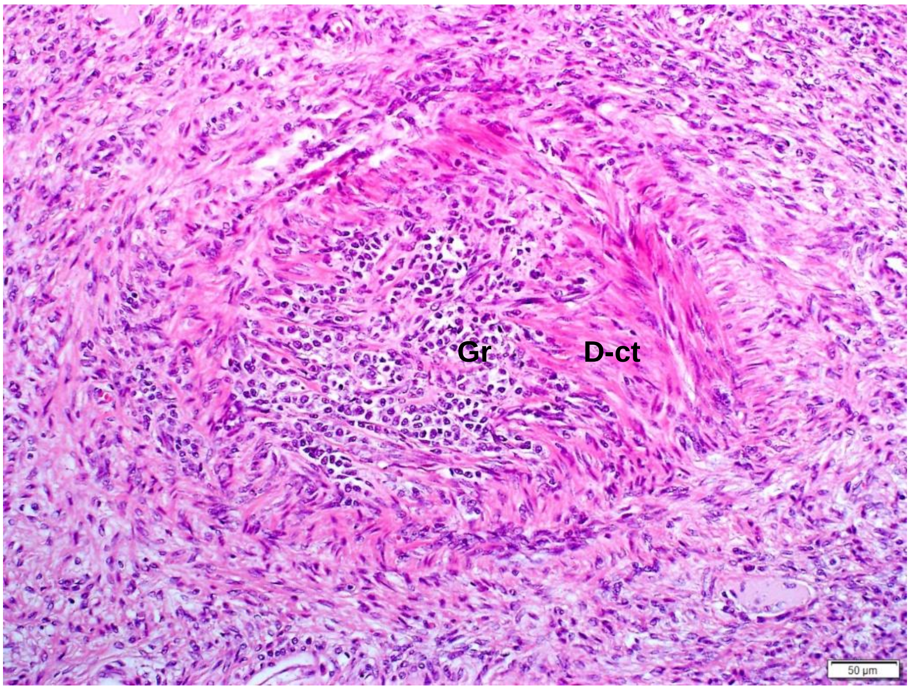


Figure 8.47: Moderate atresia. Dense connective tissue (D-ct) completely fills the former antrum replacing the remaining granulosa cells (Gr). A glassy membrane does not form in smaller atretic follicles like this one. Bar = 50 µm.

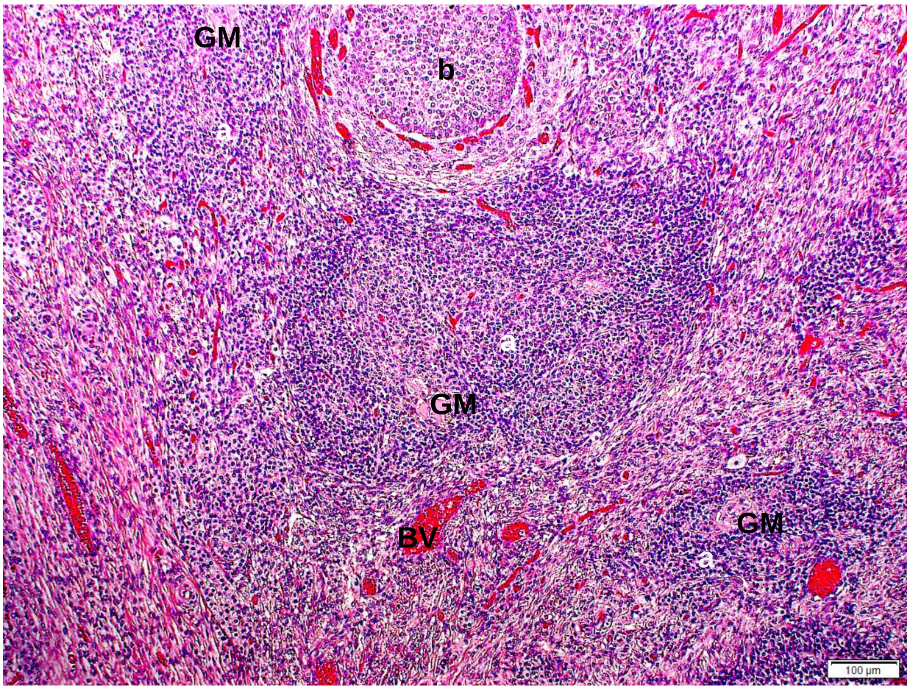


Figure 8.48: Moderate atresia of three adjacent follicles (a). Dense connective tissue with blood vessels (BV) is invading and replacing the granulosa cells. The glassy membrane (GM) has almost completely regressed. Advanced early atresia of another follicle (b). Notice the difference in vascularity between different stages of atresia in ‘a’ and ‘b’. Regression of blood vessels is advancing in moderate atresia. Bar = 100 µm.

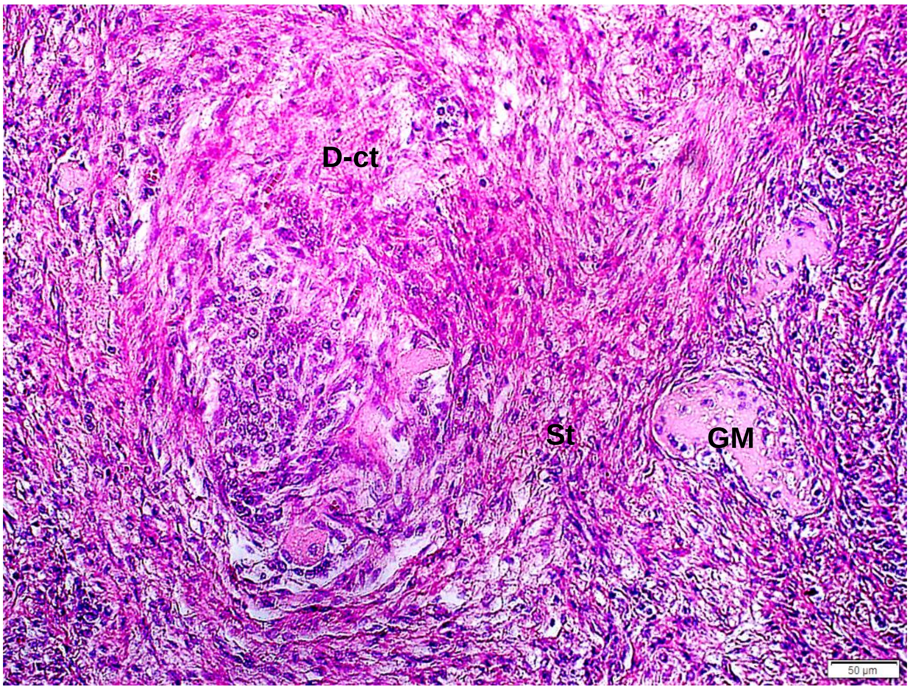


Figure 8.49: Late atresia. Dense connective tissue (D-ct) is replaced by stroma (St). The glassy membrane is not present, either having never formed in smaller follicles or having regressed during moderate atresia. Adjacent glassy membrane remnants are undergoing resorption (GM). The blood supply has regressed. Bar = 50 µm.

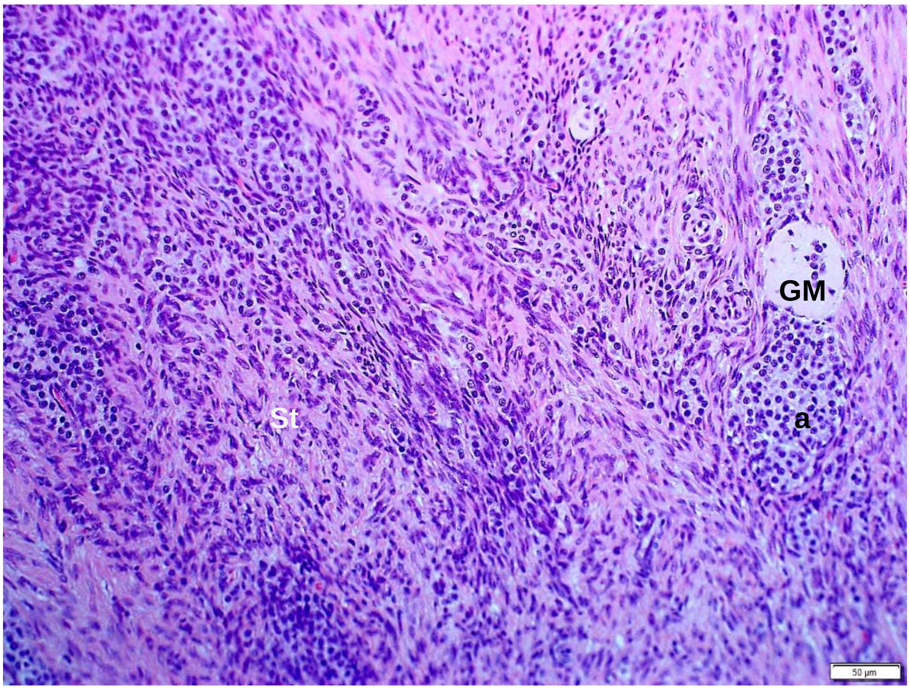


Figure 8.50: Late atresia. Normal stroma (St) replaces the connective tissue and remaining cells of the atretic follicle. A small follicle in moderate atresia (a) with its remnant of glassy membrane (GM) is visible to the right. Bar = 50 µm.

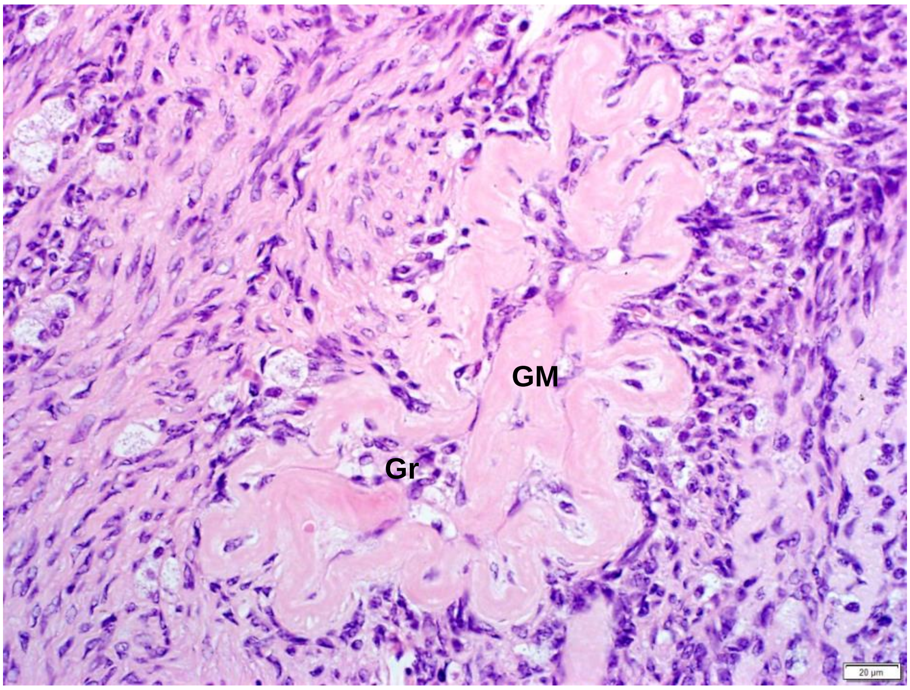


Figure 8.51: Late atresia in a 10 year-old cheetah. Occasional atretic follicles show extensive folding of the glassy membrane (GM). The granulosa cells (Gr) have been resorbed and few fibroblasts have invaded the glassy membrane. This represents an atypical scenario in the cheetah. This photomicrograph represents only a single occurrence in 70 sections (of 14 ovaries) examined. The folding of the glassy membrane and granulosa cells present in Figure 8.42 & Figure 8.43 represents the typical atretic response in the majority of cheetahs. Bar = 20 µm.

8.4. Uterine Tube

8.4.1. Gross Anatomy

The uterine tube consisted of four parts: A straight funnel shaped infundibulum with fimbriae associated with the abdominal ostium; a progressively tapering convoluted ampulla; a thinner straighter but still convoluted and tapering isthmus; with an associated straight uterine part. The funnel shaped uterine tube extended from the ovarian bursa to the apex of the uterine lumen. The uterine tube was widest at the infundibulum and tapered evenly to its termination with the uterine horn. The uterine tube ran in the mesosalpinx accompanied by a small artery and vein which was associated with the uterine tube from the proximal ampulla, at the tubal extremity of the ovary, to the junction of the isthmus with its uterine part (Figure 8.52).

From the abdominal ostium of the infundibulum the uterine tube tracked cranially, curved dorsolaterally at the level of the tubal extremity of the ovary and then ran caudally, in a medioventral direction, in the mesosalpinx to the dorsal tip of the uterine horn. It entered the uterine horn a few millimetres caudal to the outer cranial extent of the uterine horn just lateral to the attachment of the broad ligament (Figure 8.53). The uterine part of the isthmus followed a straight path caudoventrally, at 50° to the horizontal, to enter the tip of the uterine lumen at its most cranial extent. The uterine ostium opened via a microscopic papilla which could not be macroscopically appreciated.

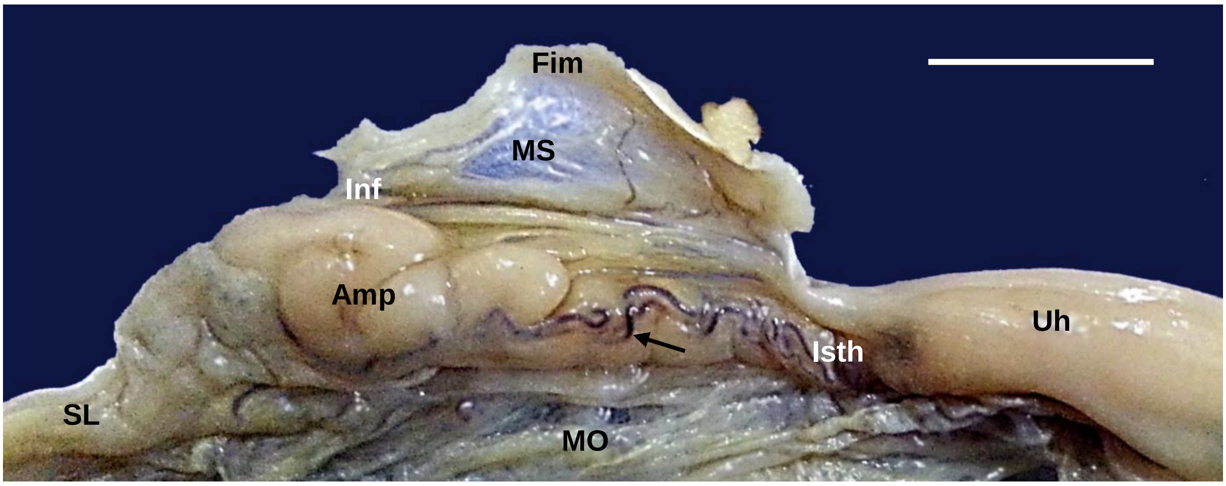


Figure 8.52: Lateral view of the right ovary of a 10.5 year-old cheetah covered by the ovarian bursa which has been partially reflected. Cranial is left. The uterine tube tapers evenly from the infundibulum to the isthmus. Notice the blood vessels (arrow) running on the uterine tube. Suspensory ligament (SL); Ampulla (Amp); Infundibulum (Inf); Fimbriae (Fim); Mesosalpinx (MS); Tip of the uterine horn (Uh); Mesovarium (MO). Bar = 10 mm.

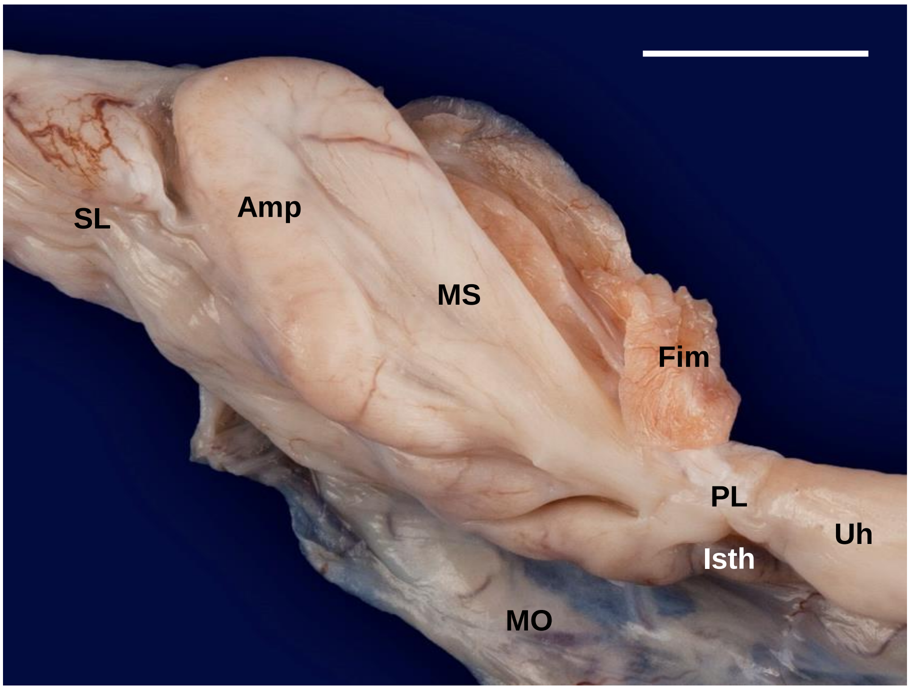


Figure 8.53: Lateral view of the right ovary of a 3 year-old cheetah covered by the ovarian bursa. Cranial is left. The uterine tube tracks cranially, curves dorsolaterally at the level of the tubal extremity of the ovary and then runs caudally in a medioventral direction in the mesosalpinx to the tip of the uterine horn (Uh). Suspensory ligament (SL); Ampulla (Amp); Mesosalpinx (MS); Fimbriae (Fim); Proper ligament (PL); Isthmus (Isth); Mesovarium (MO). Bar = 10 mm.

8.4.2. Ovarian bursal structures

The ostium of the ovarian bursa was delineated by the mesovarial free margin, the ovarian bursal attachments to the free margin of the suspensory ligament cranially and free margin of the proper ligament or tip of the uterine horn caudally, and the intervening free margins of the ovary and ligamentous structures. The opening was located medial to the ovary and the mesosalpinx extended from free margin of the ovarian bursa ventrally, laterally, dorsally and medially around the ovaries to attach to the lateral surface of the mesovarium. In 4 of 7 cheetahs (cheetahs 1&5-7) the opening of the ovarian bursa varied in size due to differing cranial or caudal attachment sites of the mesosalpinx. In 3 of 7 cheetahs (cheetahs 2-4) the mesosalpinx attachment sites were the same for both left and right ovarian bursal openings. In 2 of 14 ovarian bursal openings the cranial mesosalpinx attachment was far (>5 mm) cranial on the suspensory ligament free margin (cheetah 2)(Figure 8.54 C*).* In 7 of 14 ovarian bursal openings the cranial mesosalpinx attached 2-3 mm cranial to the tubal extremity of the ovary. In 3 of 14 ovarian bursal openings the cranial mesosalpinx attached right against the tubal pole of the ovary (Figure 8.54 A-B). In 10 of 14 ovarian bursal openings the caudal mesosalpinx attached on the free margin of the tip of the uterine horn adjacent to the attachment of the proper ligament (Figure 8.54 A&C). In 4 of 14 ovarian bursal openings the caudal mesosalpinx attached to the uterine extremity of the ovary (Figure 8.54 B; Table 8.5).

The fimbriae extended on the inner surface of the ovarian bursa dorsally, to end half way between the ovarian bursal opening and the uterine tube. Thereafter, the mesosalpinx continued as a clear sheet, with the ovary clearly visible through it, and negligible fat present (Figure 8.54 A). Blood vessels and nerves were present in the mesosalpinx.

Table 8.5: Attachment sites of the ovarian bursal opening. Cranial attachment (●); Caudal attachment (✢); Paraovarian cyst (POC).

Cheetah	Horn	Far (>5mm) cranial to the ovary on suspensory ligament	2-3mm cranial to tubal extremity of the ovary	Tubal extremity ovary	Uterine extremity of the ovary	Tip of the uterine horn
1	Right		●			✢
(3 yr)	Left			POC x 1 ●	✢	
2	Right	●	POC x 1			✢
(10.5 yr)	Left	●				✢
3	Right		POC x 1	●		✢
(10 yr)	Left			●		✢
4	Right		POC x 1 ●			✢
(10 yr)	Left		●			✢
5	Right		POC x 2 ●			✢
(10 yr)	Left			●	✢	
6	Right		●		✢	
(10.5 yr)	Left		●			✢
7	Right		POC x 1	●	✢	
(7 yr)	Left		●			✢

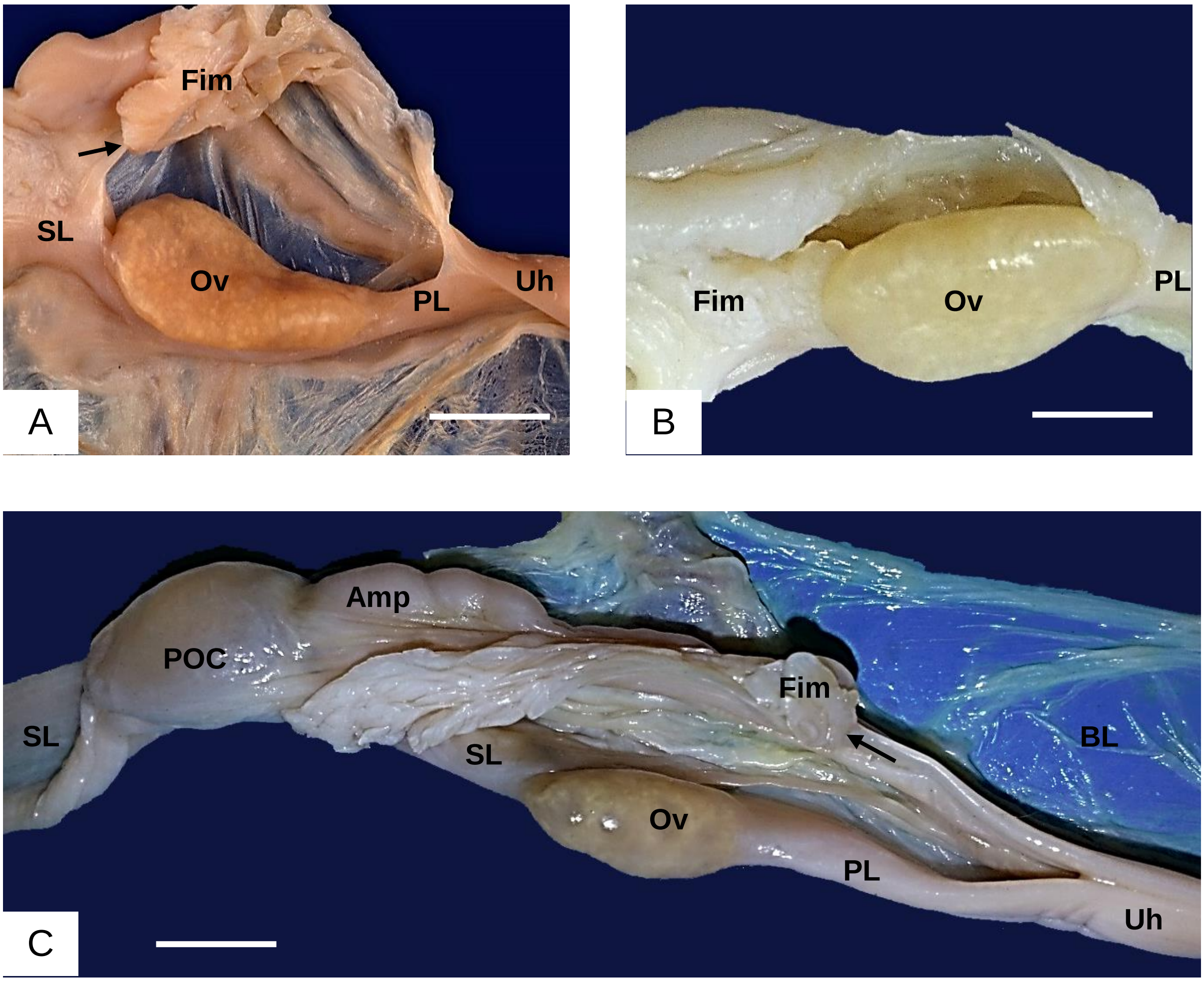


Figure 8.54: Variations in position of the attachment of the mesosalpinx to the suspensory ligament and proper ligament as well as extent of fimbrial attachment to the mesosalpinx free margin. The openings of the ovarian bursae vary in size. Cranial is left and the ovarian bursa has been reflected laterally in all images.

A: Attachment to the suspensory ligament (SL) right against the tubal extremity of the left ovary (Ov) and to the tip of the uterine horn (Uh). No fat is visible in the mesosalpinx. Fimbriae (Fim) do not extend the entire length of the free margin of the mesosalpinx (black arrow). Proper ligament (PL).

B: Attachment to the suspensory ligament cranially and proper ligament (PL) caudally, right against both extremities of the ovary (Ov) Fimbriae (Fim) extend the entire length of the mesosalpinx free margin.

C: Attachment to the suspensory ligament (SL) far (>5mm) cranial to the tubal extremity of the ovary (Ov) and to the tip of the uterine horn (Uh). A paraovarian cyst (POC) is present characteristically associated with the cranial flexure of the ampulla (Amp). Note the sudden termination of fimbriae ⅔ rd of the way along the mesosalpinx free margin (black arrow). Proper ligament (PL); Broad ligament (BL).

* Medial view of the left ovary; 3 year-old cheetah; Bar = 8mm.

** Ventral view of the left ovary; 10 year-old cheetah; Bar = 4 mm.

^ Ventrolateral view of the left ovary; 3 year-old cheetah; Bar = 5 mm.

The abdominal ostium was associated with fimbriae that were continuous with the uterine tube folds (*Plicae tubariae*) (Figure 8.57). These finger like projections and long folds consisted of strands of smooth muscle, numerous small capillaries, loose connective tissue and were covered by a ciliated and non-ciliated, simple cuboidal to low columnar epithelium

(Figure 8.55 A-B***)***. The smooth muscle bundles of the fimbrial folds that extended to the free margin of the suspensory or proper ligaments merged with the smooth muscle of those structures (Figure 8.56). Primary and secondary folds of the fimbriae were identified.

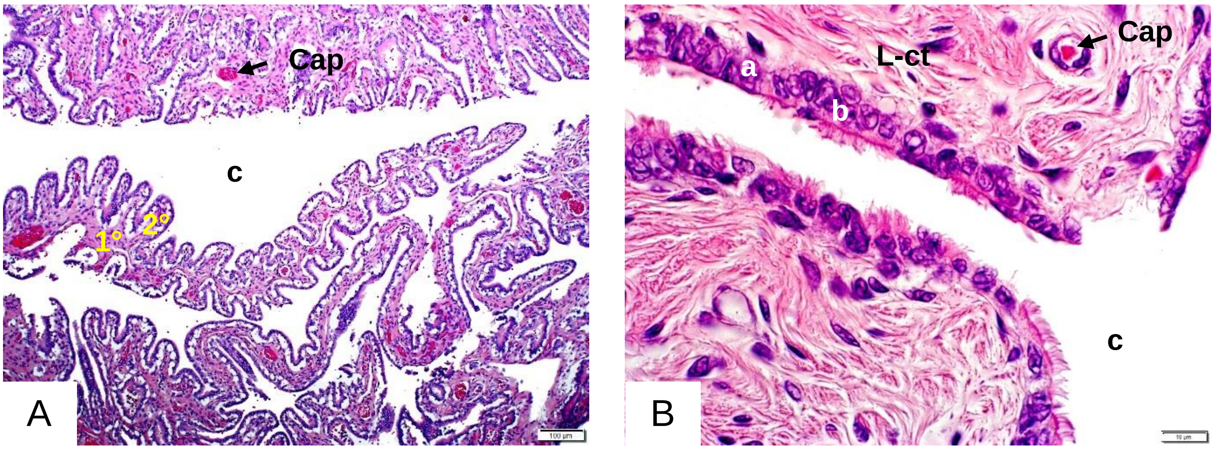


Figure 8.55: A: Fimbriae showing primary (1°) and secondary folds (2°) with a rich capillary supply (Cap). Bar = 100 µm.

B: Cuboidal to low columnar non-ciliated (a) and ciliated (b) epithelium is supported by irregular loose connective tissue (L-ct) with capillaries. Intra-bursal space (c). Bar = 10 µm.

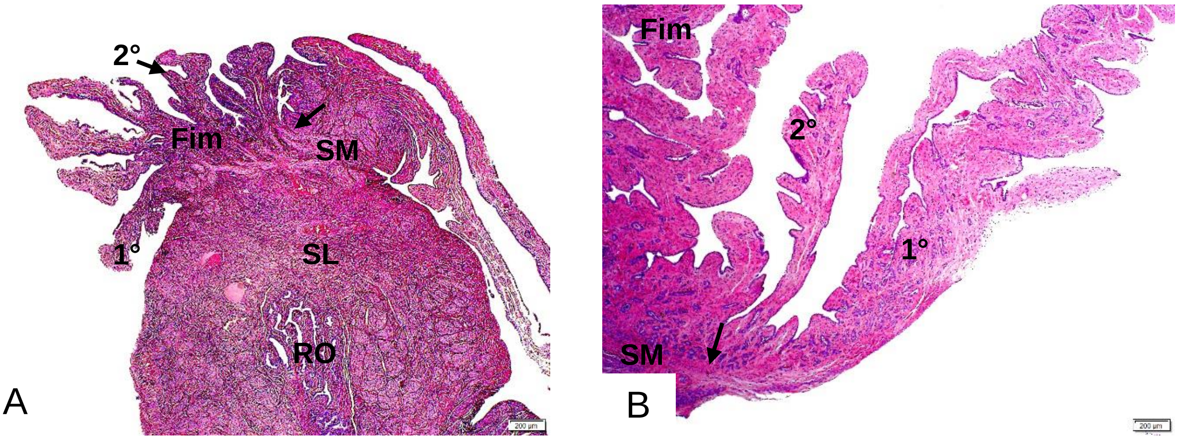


Figure 8.56: A: Transverse section through the fimbriae (Fim) at the attachment of the mesosalpinx to the suspensory ligament (SL) free margin showing primary (1°) and secondary folding (2°). The smooth muscle (SM) of the suspensory ligament extends into the fimbriae (arrow). *Rete ovarii* (RO). Bar = 200 µm.

B: A transverse section through the fimbrial attachment to the proper ligament showing the smooth muscle (SM) of the proper ligament merging with that of the fimbriae (arrow). Primary fimbrial folds (1°); Secondary fimbrial folds (2°). Bar = 200 µm.

8.4.3. Uterine tube overview

The uterine tube consisted of a mucosa-submucosal layer which formed numerous primary, secondary and tertiary folds in the infundibulum and ampulla. These folds gradually decreased in number and height from around 20 or more in the proximal uterine tube to 4-5 primary folds in the uterine part of the isthmus (Figure 8.57 B; Figure 8.59 A; Figure 8.60 A; Figure 8.63 A; Figure 8.67 A). The uterine tubal folds consisted of strands of smooth muscle and loose connective tissue, containing small blood vessels and covered in mucosa. The mucosal lining consisted of simple columnar, ciliated and secretory non-ciliated cells. The impression gained through subjective observation was that ciliated cells appeared more numerous than non-ciliated cells (Figure 8.59 A; Figure 8.61 A-B). Both of these cell types disappeared in the terminal isthmus where they were replaced by a simple cuboidal to low cuboidal epithelium (Figure 8.67 B).

The *Tunica muscularis* of the infundibulum and ampulla was uniformly thick but approximately a third thinner that of the isthmus. The inner layer of circular smooth muscle became proportionately thicker, compared with the longitudinal smooth muscle layer, in the uterine part of the isthmus where it blended with the uterine circular smooth muscle layer (Table 8.6).

Table 8.6: Mean thickness of the *Tunica muscularis* at different regions of the uterine tube in 7 cheetahs. The longitudinal smooth muscle layer thickness varied tremendously in individual measurements due to the prominent smooth muscle component present in the mesosalpinx. Where possible, measurements of the tunica muscularis were made in areas where the smooth muscle of the mesosalpinx was playing a minimal role. The inner circular smooth muscle layer provided a far more uniformly thick structure to measure.

	Circular smooth muscle layer thickness (µm)	Longitudinal smooth muscle layer thickness (µm)	Tunica muscularis thickness (µm)
Infundibulum	154.25	148.58	302.83
Ampulla	143.48	135.64	279.12
Isthmus	281.78	220.78	502.56

The smooth muscle bundles of the *Tunica muscularis* were separated into bundles by loosely arranged connective tissue. Occasional radial strands of smooth muscle from the circular smooth muscle layer of the uterine tube penetrated the mucosa-submucosal layer to infiltrate the latter's primary folds. The inner circular smooth muscle layer of the uterine tube was separated from the outer longitudinal smooth muscle layer by a vascular layer containing blood vessels and nerves. The outer longitudinal smooth muscle layer of the uterine tube produced an occasional oblique branch which merged with the inner circular smooth muscle layer of the uterine tube (Figure 8.58; Figure 8.62). The outer longitudinal smooth muscle layer of the uterine tube blended with the outer longitudinal smooth muscle layer of the uterus (Figure 8.70) and the smooth muscle of the mesosalpinx (Figure 8.22). The uterine tube was covered by a layer of serosa.

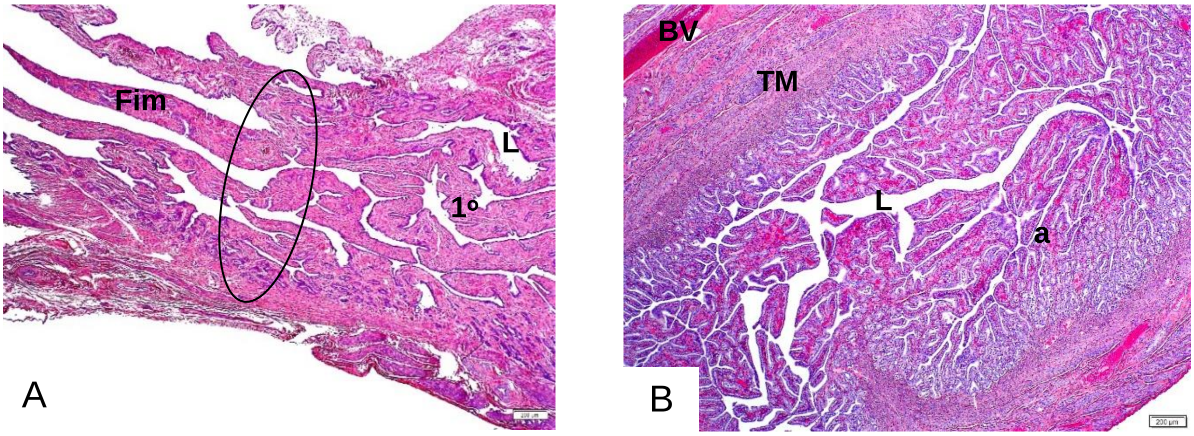


Figure 8.57: A: Longitudinal section through the entrance of the infundibulum. Primary folds (1°) are continuous with the fimbriae (Fim) at the abdominal ostium (black oval). The infundibulum continues to the right. Bar = 200 µm. B: Longitudinal section through the infundibulum with numerous mucosa-submucosal folds present (a). Lumen of the infundibulum (L); *Tunica muscularis* (TM); Blood vessels (BV). Bar = 200 µm.

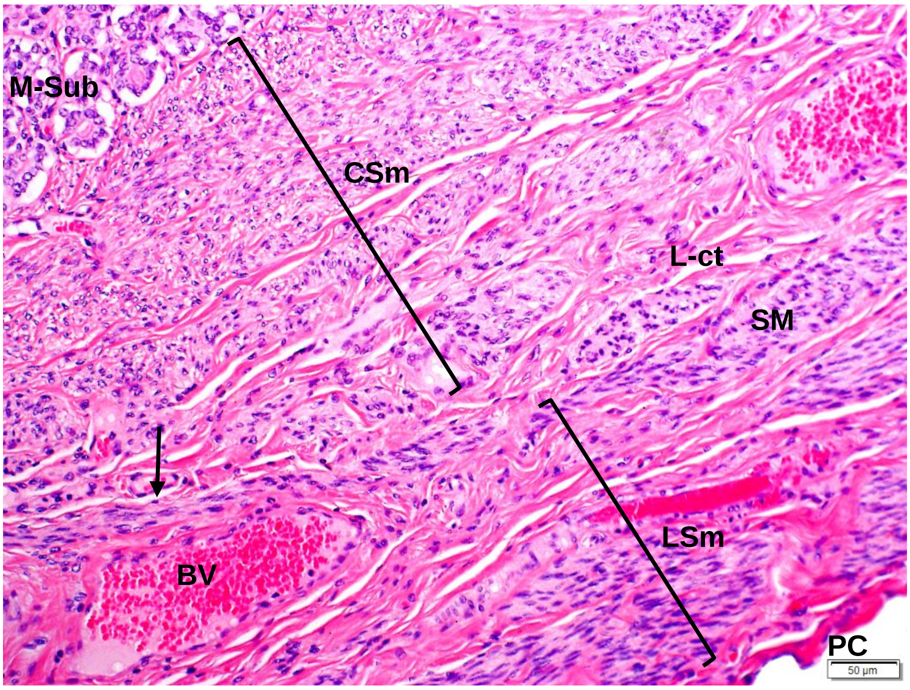


Figure 8.58: Longitudinal section through the Infundibulum. The smooth muscle layers (SM) of the *Tunica muscularis* are robust. Mucosa-submucosa (M-Sub); Circular smooth muscle layer (CSm); Longitudinal smooth muscle layer (LSm); Loosely arranged connective tissue (L-ct); Oblique smooth muscle branch between longitudinal and circular smooth muscle layers (black arrow); Blood vessels (BV); Peritoneal cavity (PC). Bar = 50 µm.

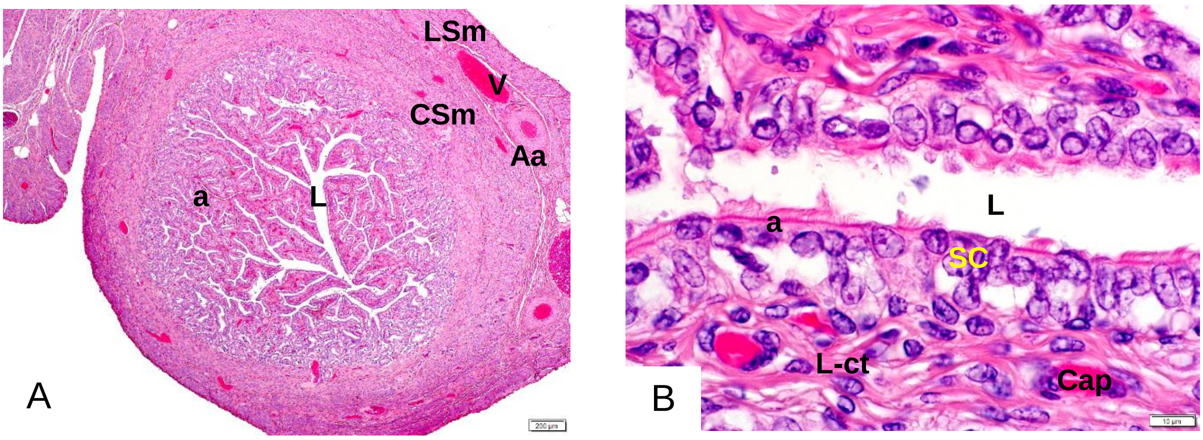


Figure 8.59: A: Transverse section through the proximal ampulla of the uterine tube. *Plicae uteriae* comprising primary, secondary and tertiary folds are present (a). Lumen of the uterine tube (L); Circular smooth muscle layer (CSm); Longitudinal smooth muscle layer (LSm); Veins (V); Arteries (Aa); Bar = 200 µm. B: Mucosa lining the lumen (L) of the proximal ampulla. Simple columnar, ciliated epithelium (a); Secretory, non-ciliated cells (SC); Loose connective tissue (L-ct); Capillaries (Cap). Bar = 10 µm.

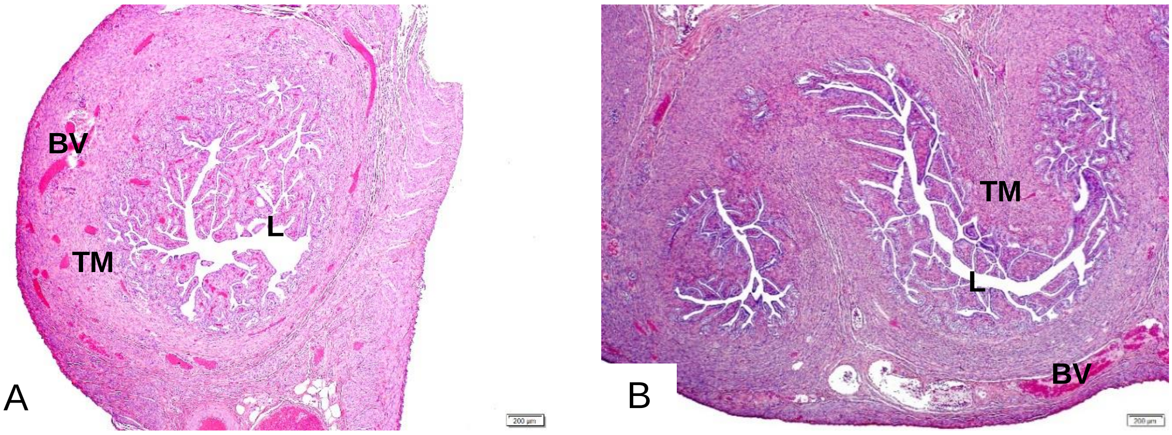


Figure 8.60: A: Transverse section (Bar = 200 µm) and B: longitudinal section through the mid ampulla of the uterine tube. The lumen size (L) and number of mucosa-submucosal folds are decreasing compared with Figure 8.59 but the thickness of the *Tunica muscularis* (TM) remains constant. Blood vessels (BV). Bar = 200 µm.

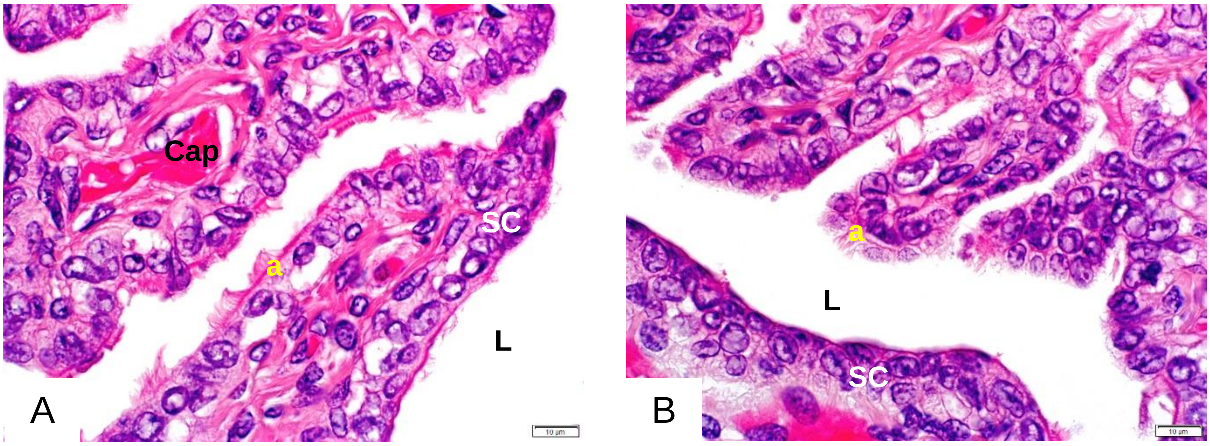


Figure 8.61: Transverse section through the mid ampulla. A: In areas ciliated simple columnar epithelium (a) predominates. Bar = 10 µm. Whereas in adjacent areas B: Non-ciliated secretory cells (SC) are more prevalent. Ciiated cells (a); Capillaries (Cap); Lumen (L). Bar = 10 µm.

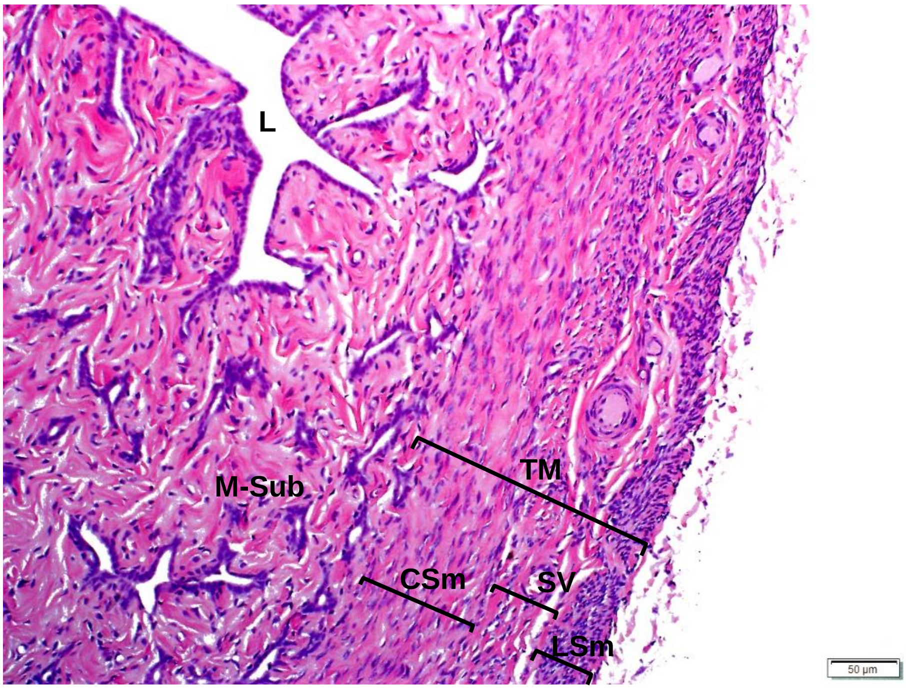


Figure 8.62: Transverse section through the middle of the ampulla. The mucosa-submucosa (M-Sub) consists chiefly of loosely arranged connective tissue which supports the epithelium lining the lumen (L); Circular smooth muscle layer (CSm); *Stratum vasculare* (SV); Longitudinal smooth muscle layer (LSm); *Tunica muscularis* (TM). Bar = 50 µm.

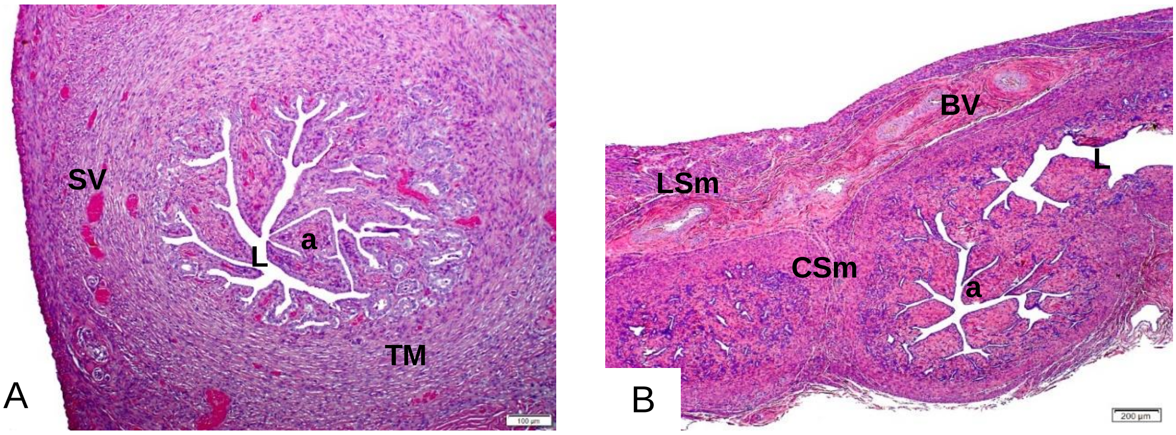


Figure 8.63: A: Transverse section (Bar = 100 µm) and B: longitudinal section through the isthmus. The lumen (L) size and number of uterine tube folds (a) are decreasing compared with Figure 8.59 & Figure 8.60. The thickness of the *Tunica muscularis* (TM) is thicker than the previous regions of the uterine tube. Notice the blood vessels that run the length of the ampulla and isthmus (BV). *Stratum vasculare* (SV); Circular smooth muscle layer (CSm); Longitudinal smooth muscle layer (LSm); Bar = 100 µm.

8.4.4. Uterine part of the isthmus and the uterotubal junction

The isthmus was convoluted, but not as extensively as the ampulla, and accompanied by a small blood vessel. The isthmus was situated in the mesosalpinx and positioned just dorsal to the proper ligament. The isthmus consisted of a convoluted extramural portion and a straight intramural uterine part which opened into the apex of the uterine horn from a microscopic papilla which could not be appreciated macroscopically (Figure 8.64; Figure 8.65). Low primarily transverse folds of endometrium encircled the papilla, loosely interdigitated with each other, restricting the uterine lumen, and extended a short distance from the apex of the uterine horn caudally (Figure 8.65).

The isthmus entered the mesometrial surface of the uterine horn at a point a few millimetres distal to the external apex of the uterine horn. The extramural portion of the isthmus consistently made a 90° bend 2-3 mm prior to entering the myometrium. At the external myometrial junction the isthmus immediately turned caudally to run at 50° to the horizontal to open in the tip of the uterine lumen from a microscopic papilla (Figure 8.64; Figure 8.66). The inner circular and outer longitudinal smooth muscle layers constituting the *Tunica muscularis* of the terminal isthmus merged with their uterine counterparts (Figure 8.68). The muscle layers were prominent. Only 4-5 primary mucosa-submucosal folds were present in the uterine part of the isthmus lined by a simple cuboidal to low cuboidal epithelium (Figure 8.67). The uterine ostium was housed in a dome shaped papilla which protruded from the dorsal roof of the apex of the uterine lumen. The papilla consisted of the inner circular smooth muscle of the isthmus, which projected through the inner circular smooth muscle of the uterus, surrounded by irregular dense connective tissue with stromal cells (Figure 8.68). Branched tubulo-alveolar glands, referred to as papillary glands from here on, were a prominent feature of the papilla. The lumens of the papillary glands opened into the uterine lumen (Figure 8.69; Figure 8.70; Figure 8.75).

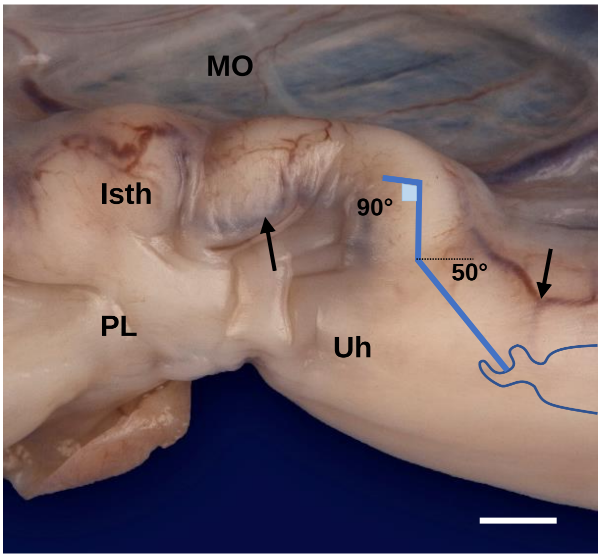


Figure 8.64: Macroscopic photograph of the isthmus and uterotubal junction. Cranial is to the left. Lateral view of the isthmus (Isth) as it enters the tip of the uterine horn (Uh). The isthmus made a characteristic 90° bend before entering the uterine horn where it bent caudally traversing the myometrium at 50° to open in a papilla. The blue overlay represents the lumens of the caudal section of the isthmus, intrauterine part of the isthmus, papilla and uterine luminal margin. Blood vessels can be seen accompanying the isthmus and anastomosing with the uterine blood vessels (arrows). Proper ligament (PL) Mesometrium (MO). Bar = 2 mm.

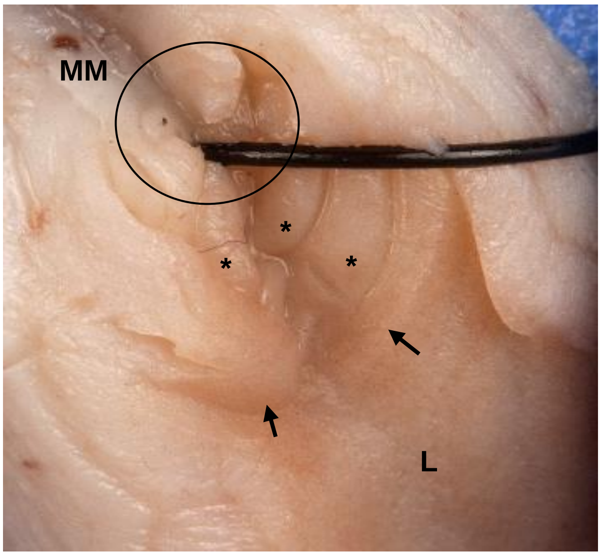


Figure 8.65: Photograph of the uterotubal junction opened longitudinally from the antimesometrial side of the uterus. The uterine lumen (L) has been opened to its apex and the incision continued cranially into the myometrium (MM) to aid visualisation of the papilla. The isthmus opened into the uterus from a papilla (black circle) at the apex of the lumen of the uterine horn. The papilla was encircled by low, rounded ridges of endometrium (*) which guarded the uterine ostium and extended a short distance caudally before ending (black arrows). A piece of 3/0 black nylon is seen protruding from the uterine ostium and was used to correctly identify the uterine ostium which was not visible to the naked eye. The black nylon = 6 mm.

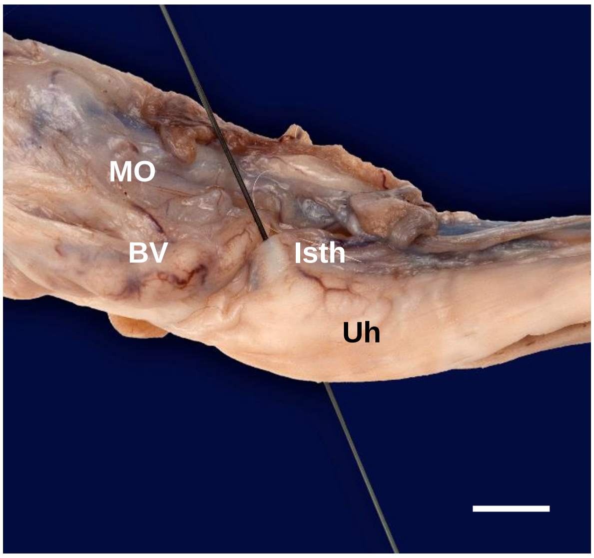


Figure 8.66: 3/0 Nylon has been passed through the transected isthmus (Isth) and out of the uterine ostium into the uterine lumen. The uterine lumen was opened from the antimesometrial surface This shows the uterine tube runs straight to the uterotubal junction at about 50° to the horizontal. Tip of uterine horn (Uh); Blood vessels (BV); Mesometrium (MO). Bar = 4 mm.

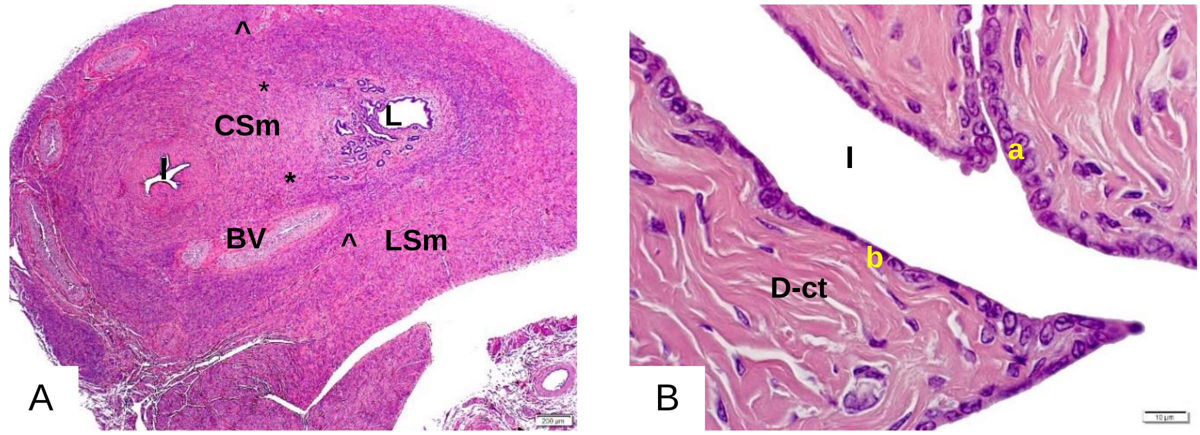


Figure 8.67: Uterine part of the isthmus.

A: Transverse section. The circular (CSm) and longitudinal smooth muscle (LSm) layers of the uterus and isthmus merge with their respective counterparts at points (*) & (^) respectively. Only 4-5 primary mucosa-submucosal folds are present in the uterine part of the isthmus. Uterine lumen (L); Isthmus lumen (l); Blood vessels (BV). Bar = 200 µm.

B: The isthmus is lined by a simple, non-ciliated, cuboidal (a) to low cuboidal epithelium (b). Dense connective tissue (L-ct); Isthmus lumen (l). Bar = 10 µm.

The uterine ostium was situated at the apex of the UTJ papilla. At the uterine ostium the simple cuboidal or low cuboidal epithelium lining the uterine part of the isthmus abruptly transitioned to a simple columnar epithelium that covered the apex of the papilla (Figure 8.79). The outer surface and shape of the papilla was generally smooth and symmetrical although some folding of the mucosa-submucosa was distinctly present in the region of the uterine ostium. Variation in this regard was evident between cheetahs (Figure 8.68; Figure 8.69; Figure 8.70; Figure 8.75). The base of the papilla was associated with concentrations of dilated papillary glands (Figure 8.68; Figure 8.75). About halfway from the apex to the base of the papilla the columnar epithelium transitioned abruptly to a simple cuboidal or low cuboidal epithelium (Figure 8.73; Figure 8.74) consistent with the rest of the uterine lumen. Thus the columnar cells formed a sharply demarcated zone encircling the uterine ostium and covering the apex of the papilla (Figure 8.76; Figure 8.77; Figure 8.78).

Branched tubulo-alveolar glands were present from the apex to the base of the UTJ papilla and did not penetrate the circular smooth muscle of the uterine tube. The papillary glands opened into the uterine lumen (Figure 8.71). The papillary glands were generally lined by epithelium resembling the surface epithelium of the papilla closest to them, namely simple columnar epithelium in the apical region and simple cuboidal epithelium nearer the base of the papilla. Some glands presented a squat profile while others were long and narrow in appearance (Figure 8.71; Figure 8.72). In some sections the glands appeared to concentrate at the base of the fornix. The point of transition between the papillary glands and uterine glands differed between cheetahs. In some cheetahs the papillary glands were restricted to the base of the fornix (Figure 8.69), while in others they extended a short distance beyond the base into the endometrium of the uterus (Figure 8.68). The interface between the two types of glands was not always clearly defined. The papillary glands often appeared dilated to varying degrees and this was especially evident at the base of the fornix (Figure 8.68; Figure 8.70; Figure 8.75) In one 10.5 year-old cheetah the papillary glands at the base of the fornix were grossly dilated forming cyst-like structures, some of which were filled with proteinaceous fluid. The endometrial lining of the fornix was distinctly narrower than that of the uterine horn (Figure 8.75).

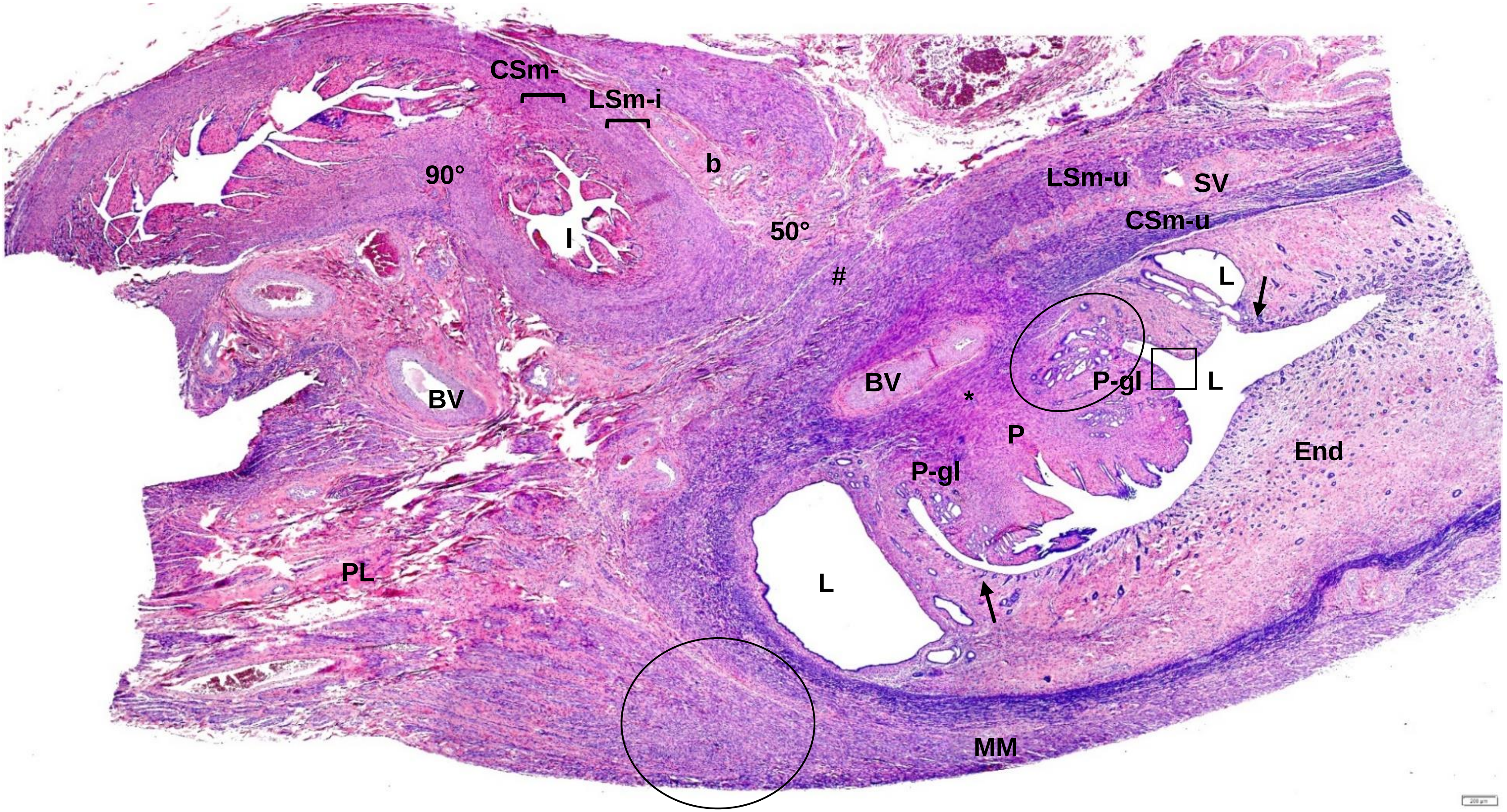


Figure 8.68: Longitudinal section through the isthmus of a 10 year-old cheetah showing it entering the external uterine horn and opening in the uterine lumen via a papilla (P). The papilla is situated a few millimetres caudal to the external junction of the isthmus with the uterine horn. The proper ligament (PL) merges (black circle) with the longitudinal smooth muscle layer of the myometrium (MM). The longitudinal smooth muscle layer of the isthmus (LSm-i) merges with the longitudinal smooth muscle layer of the uterus (LSm-u) at point #. The circular smooth muscle of the isthmus (CSm-i) merges with the circular smooth muscle of the uterus (CSm-u) but continues into the base of the papilla (*). The *Stratum vasculare* of the uterus (SV) is continuous with the blood vessels accompanying the uterine tube (bv). The papillary glands (P-gl) are clearly different to those of the endometrium (End) and some of the papillary gland lumens are dilated. This is especially prominent in the region of the fornix of the papilla (black oval). The black arrows indicate the appearance of the first uterine glands. Isthmus lumen (l); Isthmus 90° bend (90°) and 50° bend (50°). The black square is representative of the area examined in Figure 8.71. Bar = 200 µm.

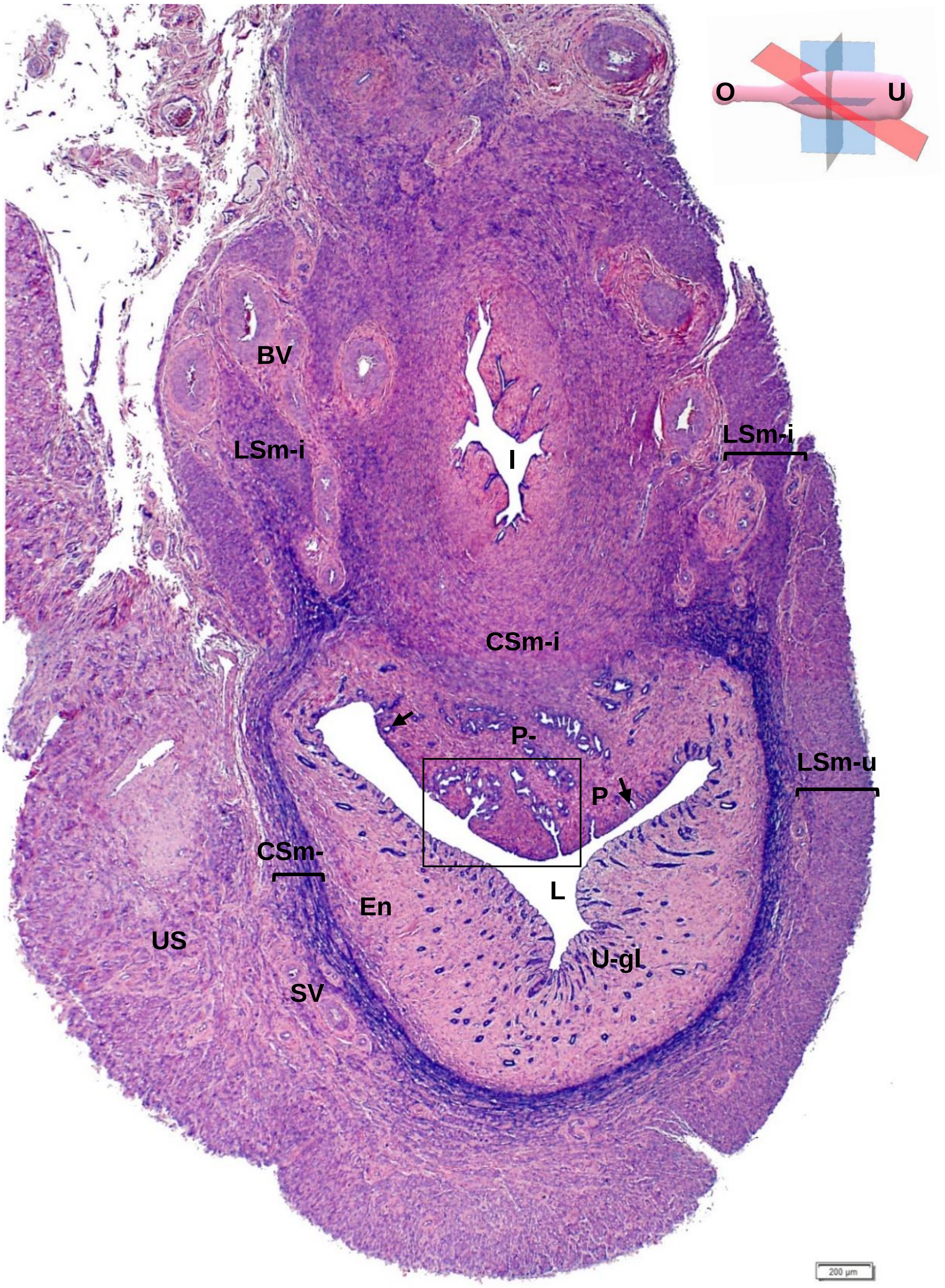


Figure 8.69: A tangential transverse section through the intramural isthmus of a 10 year-old cheetah highlighting the dome shaped papilla (P) and its associated branched tubulo-alveolar glands (P-gl). The longitudinal smooth muscle layer of the myometrium is thickened on the left as result of the smooth muscle fibres of the proper ligament augmenting it to form the uterine sling (US). A vascular plexus (BV) is present on either side of the isthmus between its circular smooth muscle (CSm-i) layer and its longitudinal smooth muscle layer (LSm-i). The glands of the papilla differ to the endometrial glands (U-gl). The black arrows indicate the appearance of the first uterine glands. Isthmus lumen (l); Uterine lumen (L); Endometrium (End); *Stratum vasculare* (SV). Circular smooth muscle of the uterus (CSm-u); Longitudinal smooth muscle layer of the uterus (LSm-u). The black square outlines the area examined in Figure 8.71 & Figure 8.72. The coloured inset illustrates the plane of section in red (Ovary (O); Uterus (U)). Bar = 200 µm.

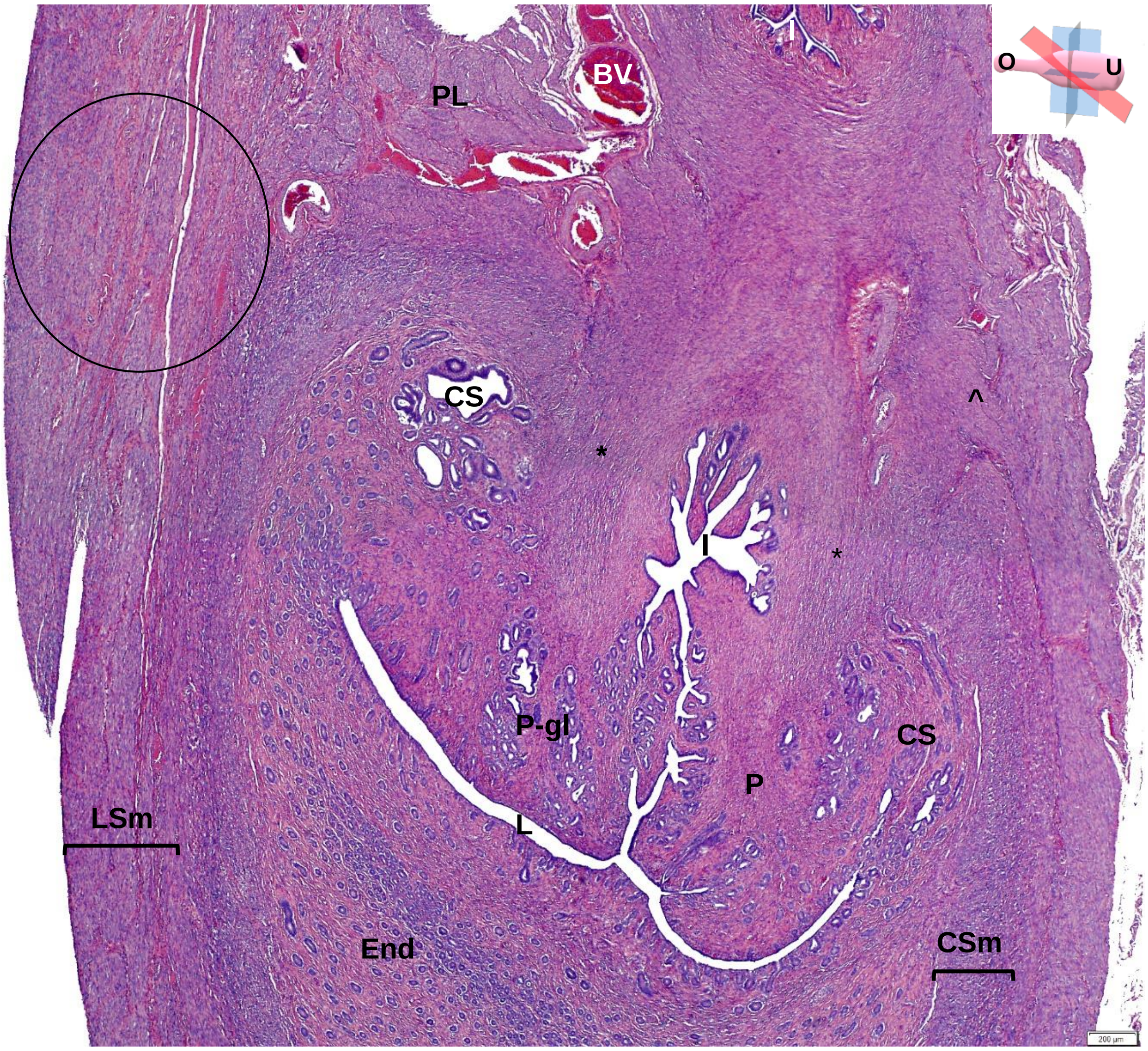


Figure 8.70: Longitudinal tangential section through the uterotubal junction of cheetah 6, age 10.5 yr. The smooth muscle layers, both circular (CSm) and longitudinal (LSm), of the isthmus and uterine horn merge with each other at points (*) and (^) respectively. In addition, the longitudinal smooth muscle layer of the uterine horn merges (black circle) with the proper ligament smooth muscle (PL). The circular smooth muscle of the isthmus can be followed to where the isthmus lumen (I) is again transected at the top of the picture. The papillary glands (P-gl) appear dilated in areas forming cystic appearing structures (CS) which are prevalent in the area of the fornix. Papilla (P); Uterine lumen (L); Endometrium (End); Blood vessels (BV); Bar = 200 µm.

Figure 8.71: Branched tubulo-alveolar papillary glands in the papilla (P). Enlargement of the area delineated by the black square in Figure 8.69. The papillary glands presented either a short, squat profile (A) or a thinner, elongated profile (B). Uterine lumen (L); Endometrium (End) with simple tubular uterine glands. Bar = 50 µm.

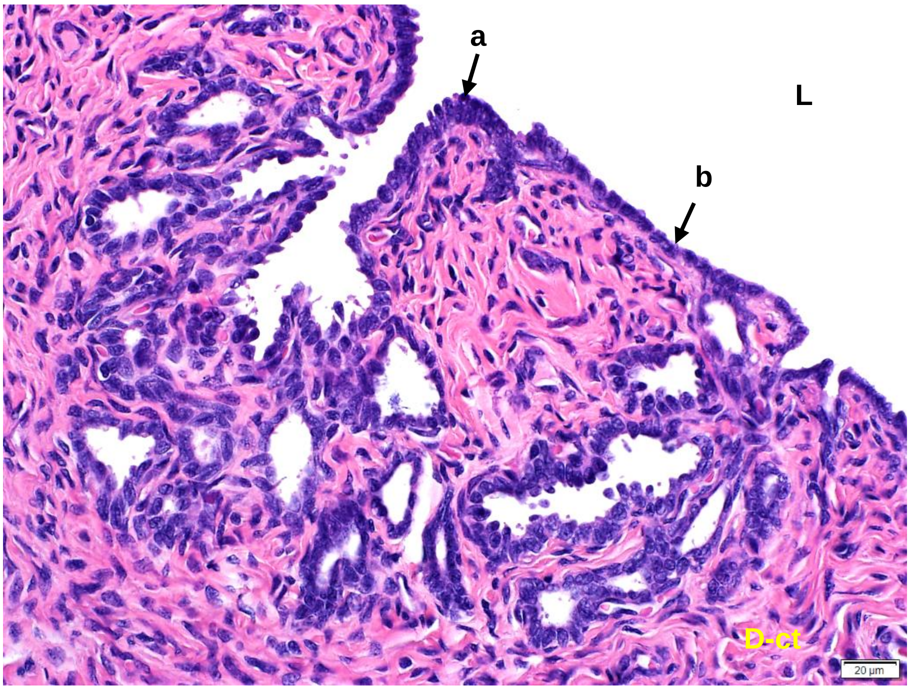


Figure 8.72: Branched tubulo-alveolar papillary glands with a squat profile lined by simple low columnar epithelium (a). Enlargement of the area delineated by the black square in Figure 8.69. Uterine lumen (L). Dense, irregularly arranged connective tissue (D-ct) of the papilla. Simple cuboidal epithelium (b). Bar = 20 µm.

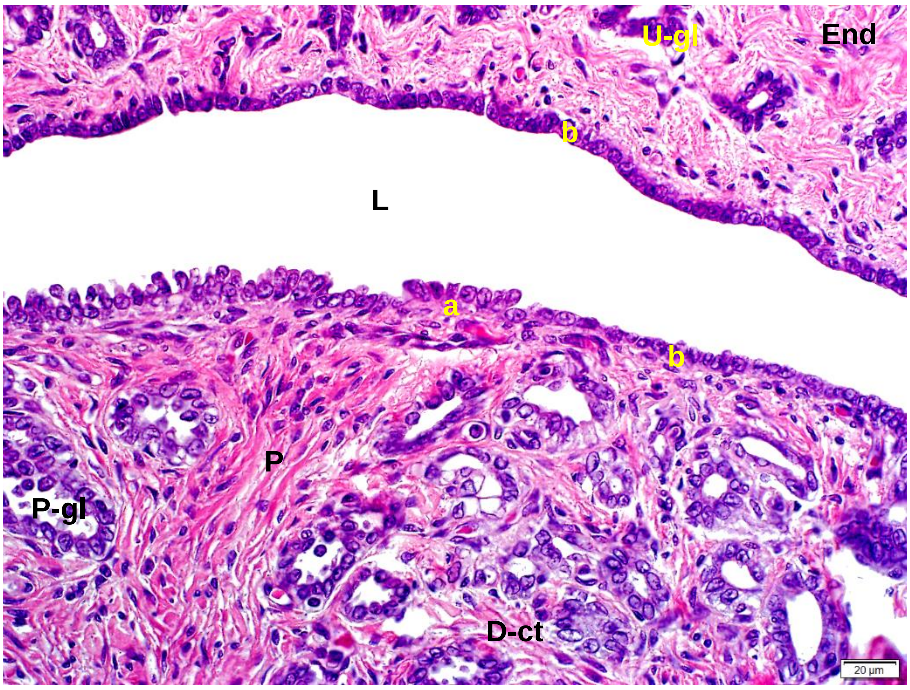


Figure 8.73: Transition of the mucosa on the papilla (P). Papillary glands (P-gl) are lined by epithelium similar to the surface of the papilla closest to them. Notice the difference in this regard between the glands on the left and right of the picture. Uterine lumen (L); Simple columnar epithelium (a); Simple cuboidal epithelium (b); Uterine Glands (U-gl); Dense connective tissue (D-ct); Endometrium (End). Bar = 20 µm.

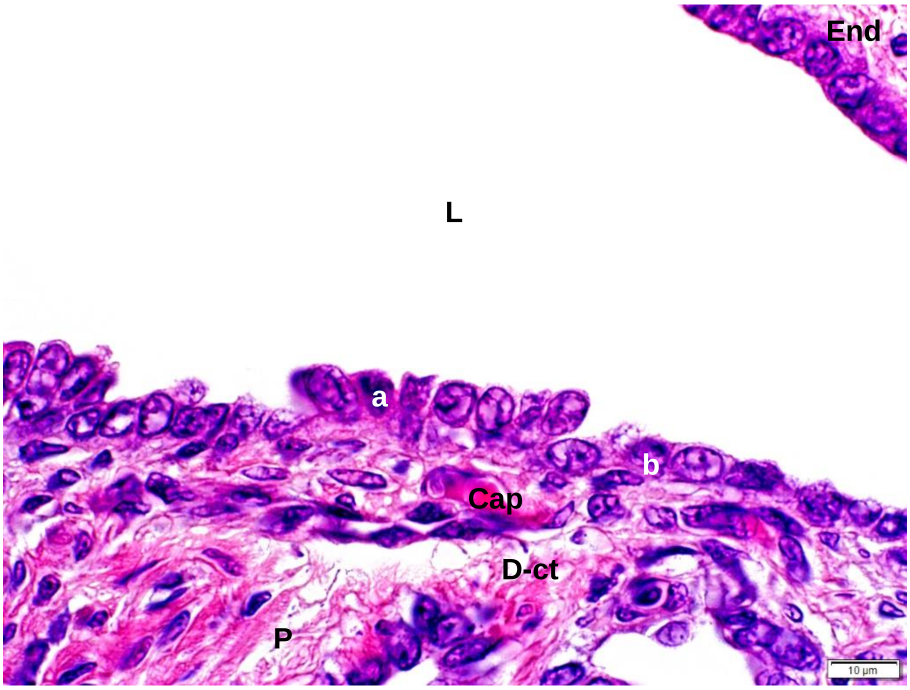


Figure 8.74: An abrupt transition from simple columnar (a) to simple cuboidal (b) epithelium occurs about halfway down the papilla (P). Uterine lumen (L).); Capillary (Cap); Dense connective tissue (D-ct); Endometrium (End); Bar = 10 µm.

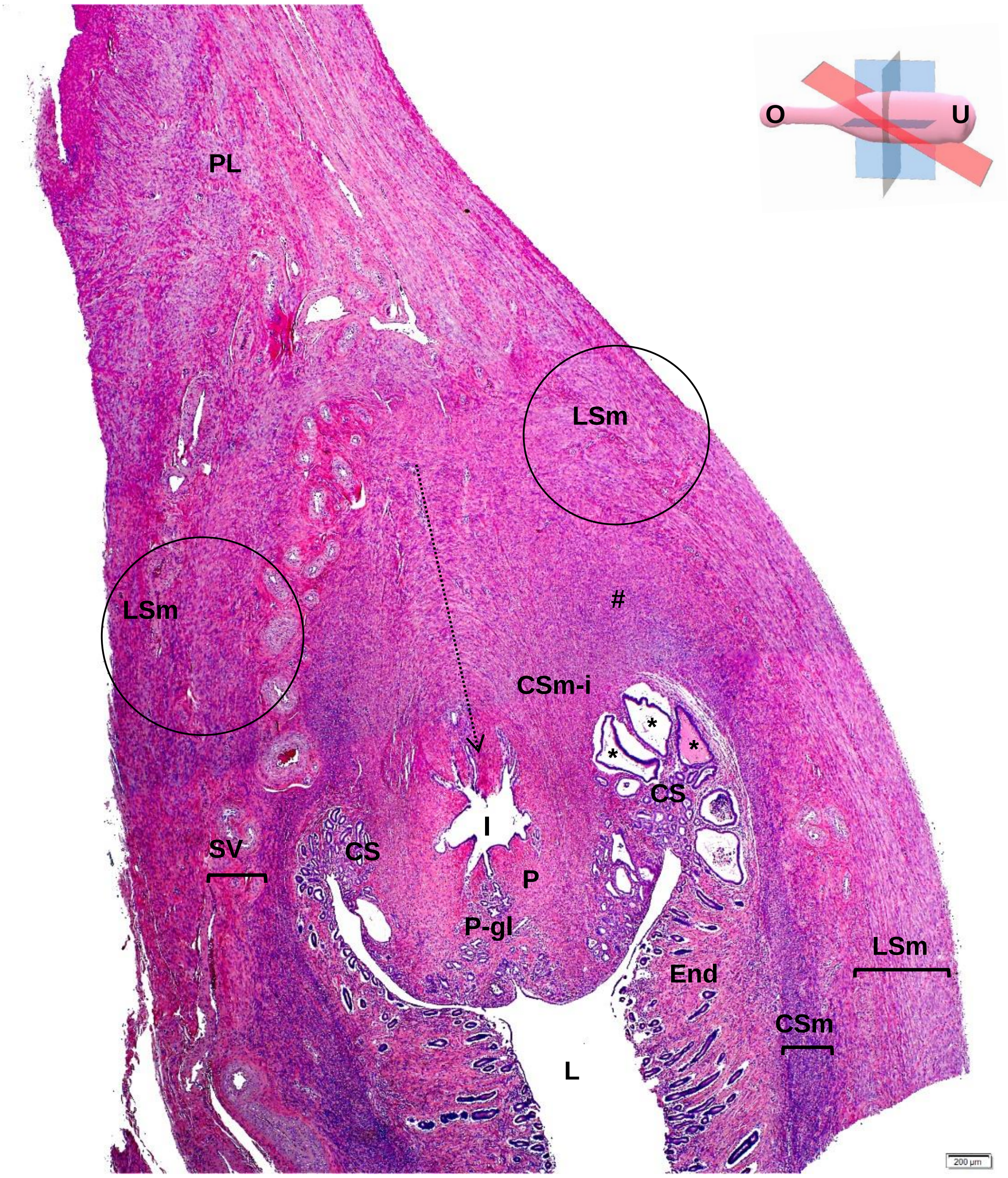


Figure 8.75: Longitudinal section through the junction of the proper ligament and isthmus to the tip of the uterine horn of a 10.5 year-old cheetah. The circular smooth muscle layer of the uterine part of the isthmus can be seen traversing the myometrium in a straight line (black dotted arrow) towards the apex of the papilla (P). The smooth muscle of the proper ligament (PL), isthmus and uterine horn blend with each other (black circles). The proper ligament is continuous with the outer longitudinal smooth muscle (LSm) layer of the uterine horn as is the longitudinal smooth muscle of the isthmus. The inner circular smooth muscle layer of the isthmus (CSm - i) blends (#) with its counterpart in the uterus (CSm). Some of the papillary glands (P-gl) in the fornix of the papilla appear to be cystic (CS) and a pink proteinaceous fluid is present in the lumen of one of the glands with traces in the other adjacent dilated glands (*). The lining of most of the dilated papillary glands has contracted away from the surrounding tissue and is considered artefactual. The endometrial lining of the fornix is distinctly narrower than that of the uterine horn. *Stratum vasculare* (SV); Lumen of the isthmus (I); Uterine lumen (L); Endometrium (End); Bar = 200 µm.

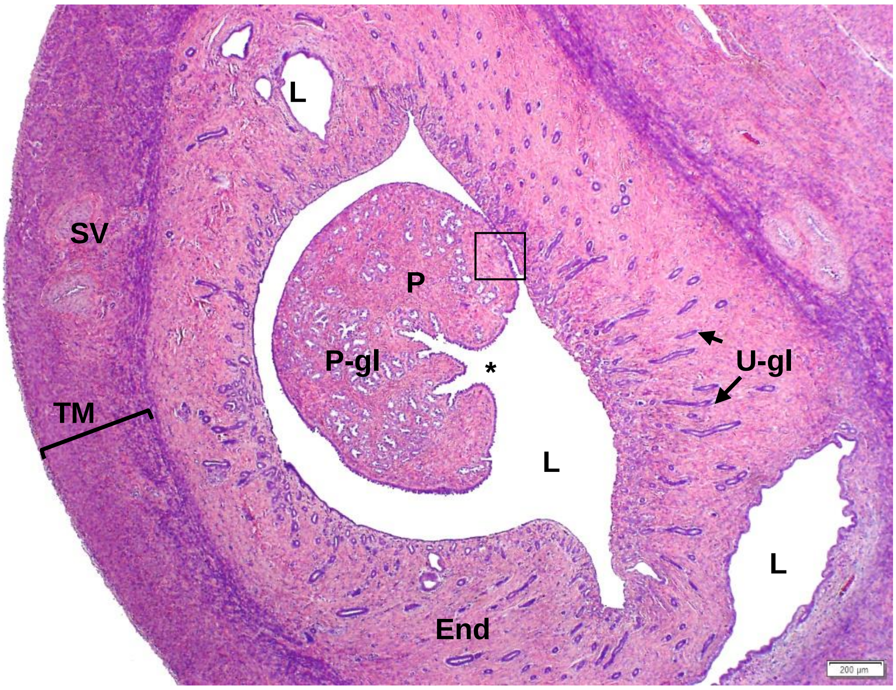


Figure 8.76: Transverse section through the isthmus papilla (P). The papillary glands (P-gl) are distinct to the uterine glands (U-gl). The glands of the papilla, its surface and the uterine ostium (*) have a different epithelium to the endometrium (End). The black square represents the area examined in Figure 8.78. Uterine lumen (L); *Tunica muscularis* (TM); *Stratum vasculare* (SV). Bar = 200 µm.

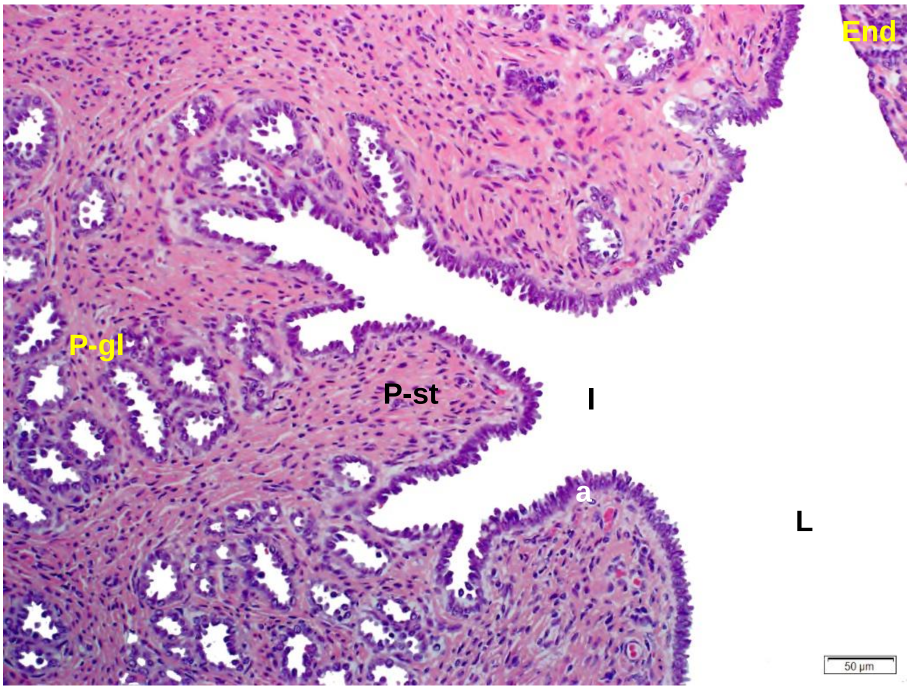


Figure 8.77: Transverse section through the papilla of the isthmus showing the uterine ostium (I) and papillary glands (P-gl) lined by a simple columnar epithelium (a). A small section of endometrium (End) covered in a simple low cuboidal epithelium is visible in the top right corner. Uterine lumen (L); Papillary stroma (P-st). Bar = 50 µm.

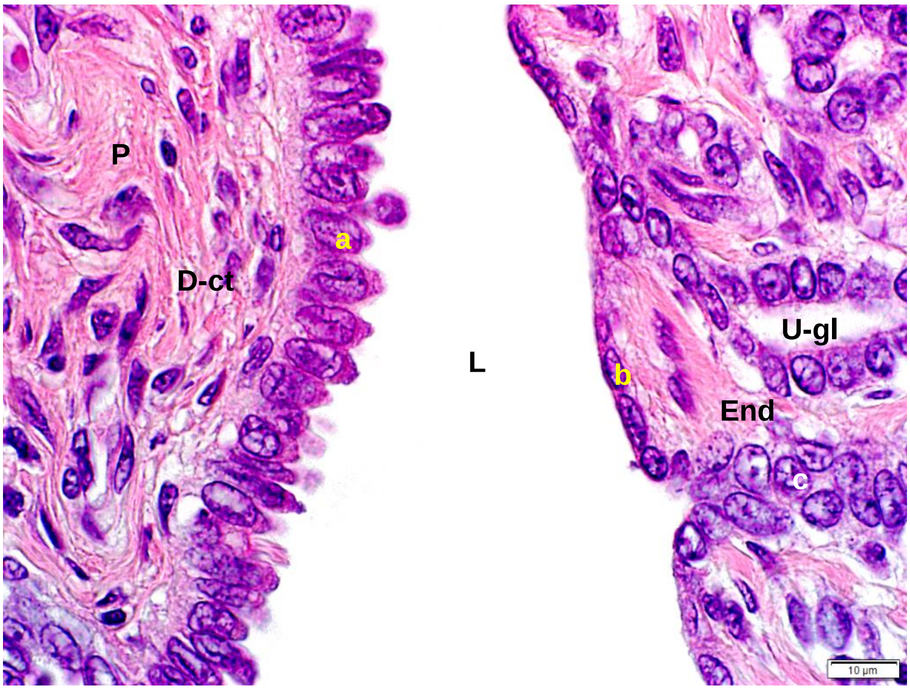


Figure 8.78: Enlargement of the area delineated by the black square in Figure 8.76. Contrast of the epithelium covering the apex of the papilla (P) on the left and the adjacent endometrium (End) to the right. Uterine lumen (L); Simple columnar epithelium (a); Simple low cuboidal epithelium (b). The uterine glands (U-gl) are lined by a simple cuboidal epithelium (c); Dense connective tissue (D-ct). Bar = 10 µm.

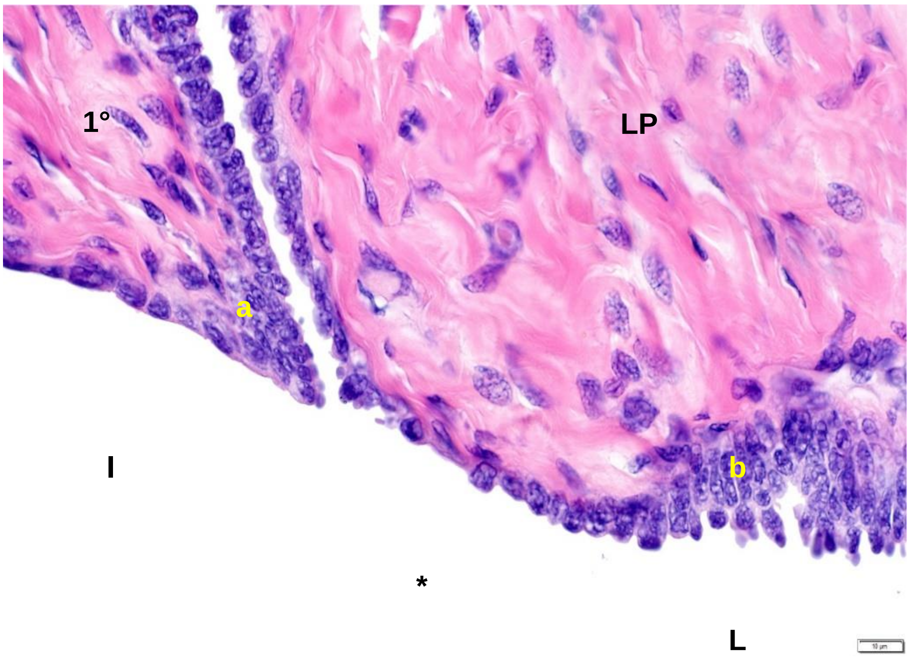


Figure 8.79: The epithelium of the uterine ostium transitions abruptly from a simple cuboidal epithelium (a) to a simple columnar epithelium (b). Irregular dense connective tissue forms a major component of the *Lamina propria* (LP). Lumen of the uterine part of the isthmus (I); Uterine ostium (*); Uterine lumen (L); Primary fold of the isthmus mucosa-submucosa (1°). Bar = 10 µm.

8.5. Uterus

The lumen of the uterus was lined by a layer of simple low cuboidal to cuboidal epithelium supported by the superficial part of the endometrium.

The endometrium consisted of two parts (*Figure 8.80*). A thin, superficial part consisted of vascular, loosely arranged connective tissue (Figure 8.81) which was rich in fibrocytes and had lymphocytes and occasional polymorphonuclear cells present. The lymphocytes were the most obvious and consistent white blood cell present. A thick deeper part consisted of dense, irregularly arranged connective tissue (Figure 8.82) that was less cellular and had more extracellular matrix than the superficial part.

The stroma consisted of simple straight or simple branched tubular glands that had a regular distribution and extended from the lumen to the myometrium. The glands were regularly distributed throughout the uterus, although some clustering of the uterine glands occurred at the poles (Figure 8.80). The uterine glands were lined by a simple cuboidal epithelium (Figure 8.81). No *Stratum compactum* was present and the *Stratum spongiosum* effectively extended from the luminal epithelium to the myometrium. A rich nerve supply was present at the junction of the endometrium with the myometrium (Figure 8.82), as were small blood vessels. Capillaries were present throughout the endometrium.

The endometrium was surrounded by the myometrium. The myometrium consisted of an inner circular layer of smooth muscle separated by a vascular layer (*Stratum vasculare*) from the outer longitudinal smooth muscle layer (*Figure 8.80*). Small blood vessels were apparent in both the circular and longitudinal smooth muscle layers. The outer longitudinal smooth muscle layer was covered by the perimetrium, a thin layer of peritoneal mesothelium. Both the perimetrium and longitudinal smooth muscle layer of the uterus was continuous with their homologous counterparts of the broad ligament. (Figure 8.83).

At the junction of the uterine horns with the uterine body, the uterine horns continued for a short distance into the uterine body as distinct structures separated by the uterine velum (Figure 8.83).

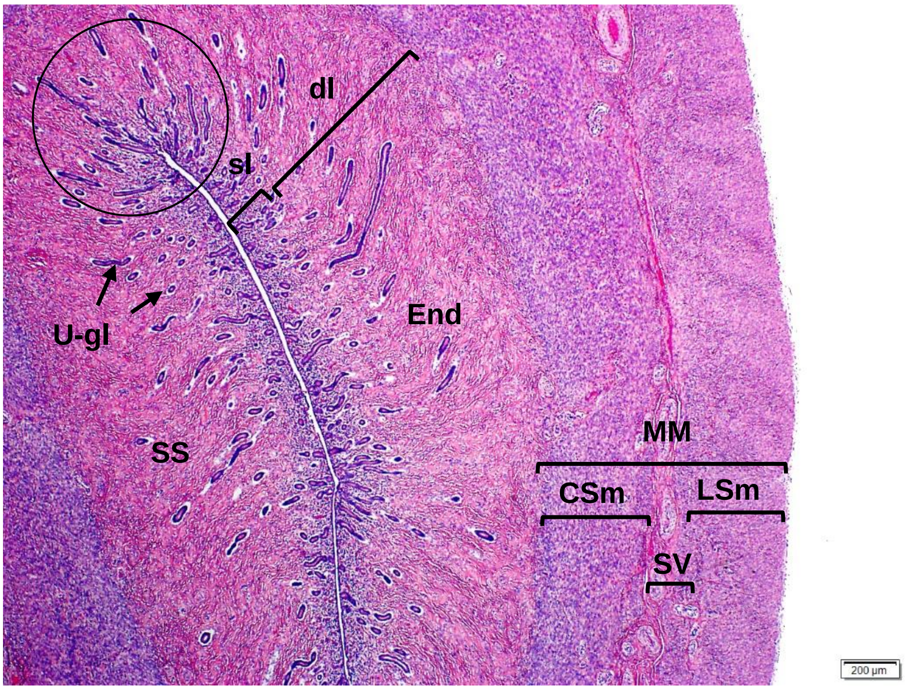


Figure 8.80: Transverse section through the uterine horn. Endometrium (End) with simple straight uterine glands (U-gl) regularly distributed, with some clustering at the uterine poles (black oval). Thin superficial layer (sl) and thick deeper layer of the endometrium (dl) comprising the *Stratum spongiosum* (SS); Myometrium (MM) with inner circular smooth muscle layer (CSm) separated from the outer longitudinal muscle layer (LSm) by the *Stratum vasculare* (SV); The perimetrium was lost during processing. Bar = 200 µm.

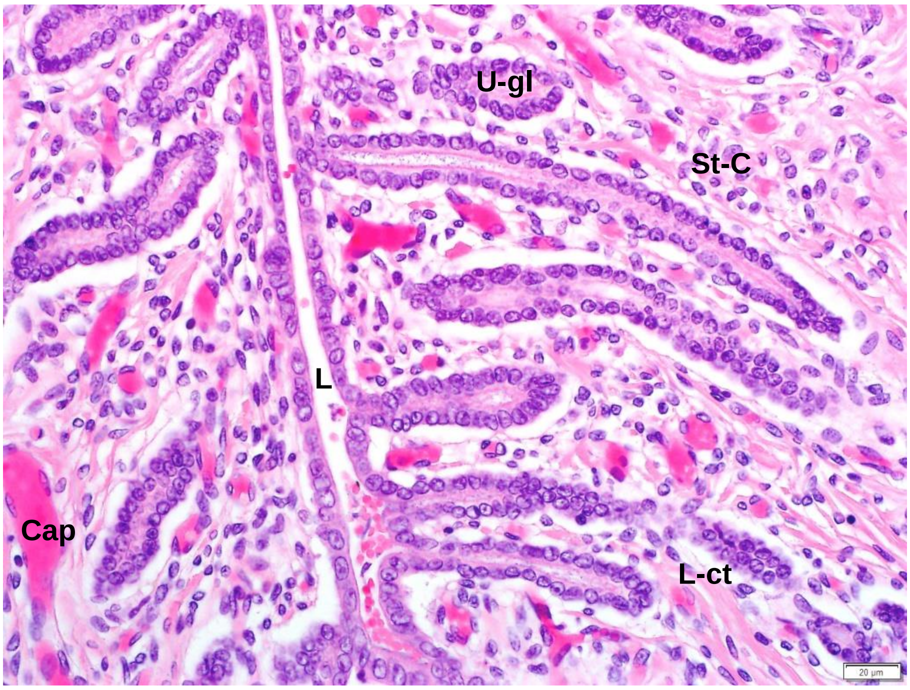


Figure 8.81: Superficial part of the endometrium. The uterine lumen (L) is lined by low cuboidal to cuboidal epithelium; The uterine glands (U-gl) are lined by cuboidal secretory epithelium. No *Stratum compactum* is visible. Capillaries (Cap), stromal cells (St-C) and loose, irregularly arranged connective tissue (L-ct) abound. Some red blood cells are present in the uterine lumen and this likely occurred during sampling. Bar = 20 µm.

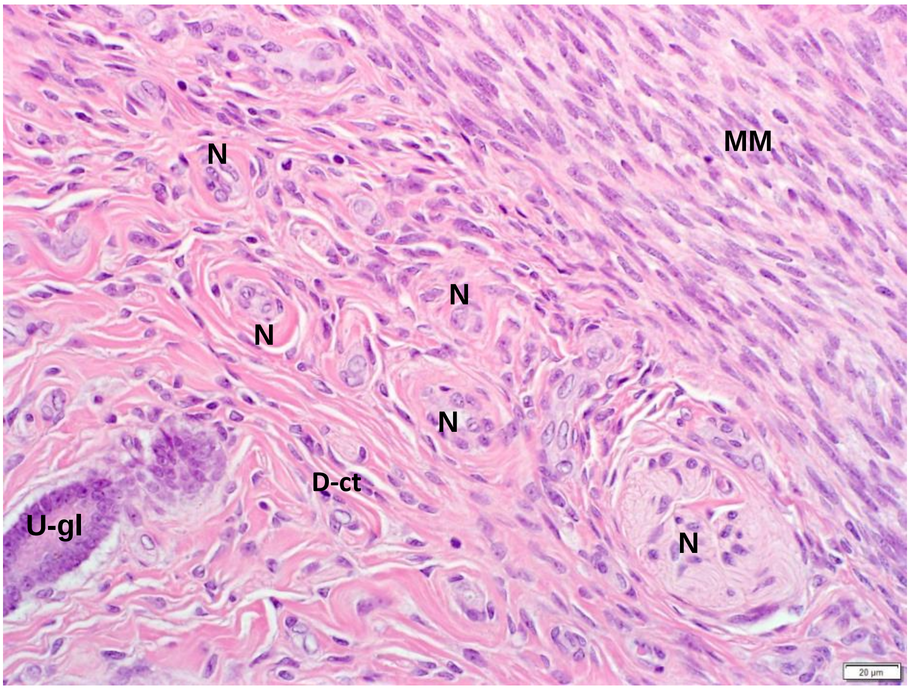


Figure 8.82: A rich nerve network is present between the endometrium and myometrium. The stroma of this deeper part of the endometrium has a prominent dense, irregular connective tissue component (D-ct) and fewer stromal cells. Myelinated nerves (N); Uterine glands (U-gl); Myometrium (MM). Bar = 20 µm.

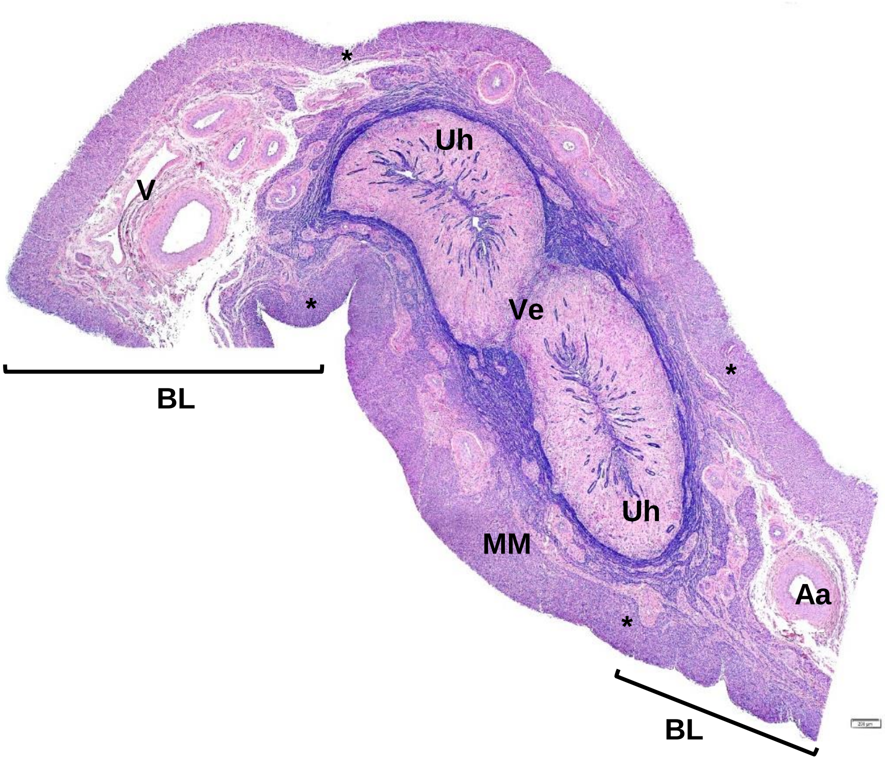


Figure 8.83: Transverse section through the proximal body of the uterus showing the uterine velum (Ve) separating the two uterine horns (Uh). The well-developed smooth muscle of the broad ligament (BL) that forms part of the uterine sling is continuous (*) with the myometrium. The intense basophilic staining of the smooth muscle was unique to this cheetah and the cause is unknown. The perimetrium was lost during processing. The broad ligament has been cut on both sides. Myometrium (MM); Arteries (Aa); Veins (V). Bar = 200 µm.

8.6. Cervix

The mucosa-submucosa consisted of a non-keratinised, non-ciliated, stratified squamous epithelium supported by the *Lamina Propria*. In places the superficial cell layer of the stratified squamous epithelium failed to flatten completely and appeared as low cuboidal cells. No mucigenous cells, goblet cells nor uterine glands were seen in the mucosa-submucosa (Figure 8.84). Mucous was not present in the cervical lumen. The *Lamina Propria* consisted of dense, irregular connective tissue which contained a blood and nerve supply. High primary folds of mucosa-submucosa with small secondary folds filled the cervical lumen. The primary folds of mucosa-submucosa consisted of smooth muscle fibres radiating from the inner circular smooth muscle layer and dense, irregular connective tissue. The *Tunica muscularis* consisted of an inner circular layer of smooth muscle and an outer longitudinal smooth muscle layer (Figure 8.85). The smooth muscle layers were continuous with the uterus cranially and vagina caudally. The *Tunica muscularis* was covered by loose connective tissue constituting the adventitia. No elastic fibres were present in any layers of the cervix (Figure 8.86 A-D).

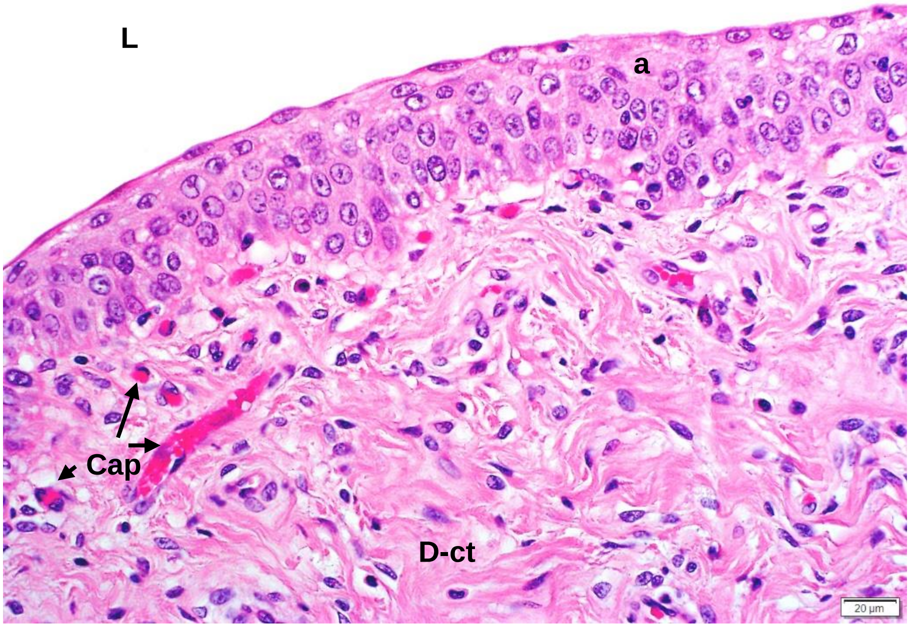


Figure 8.84: Cervical mucosa-submucosa. Some of the surface cells have failed to flatten and appear low cuboidal in places. No Goblet nor mucigenous cells are present. Dense, irregular connective tissue (D-ct); Capillaries containing blood (Cap); Non-keratinised, stratified squamous epithelium (a). Lumen (L). Bar = 20 µm

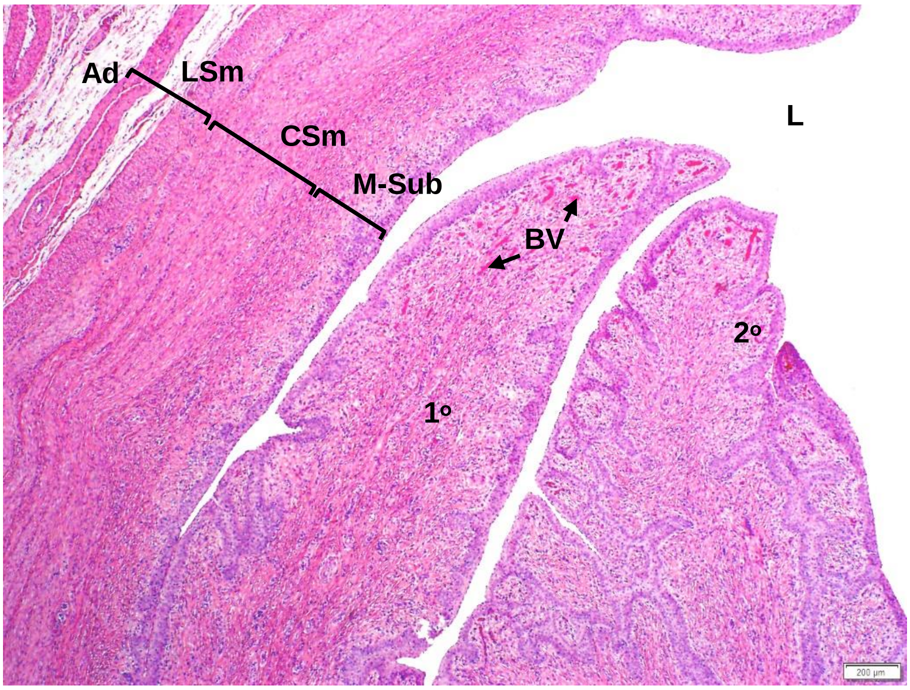


Figure 8.85: Transverse section through the cervix. No mucous is present in the cervical lumen (L). Primary folds (1º) with some secondary folds (2º) fill the lumen and have a rich blood supply (BV); Mucosa-submucosa (M-Sub); Circular smooth muscle layer (CSm); Longitudinal smooth muscle layer (LSm); Adventitia (Ad). Bar = 200 µm.

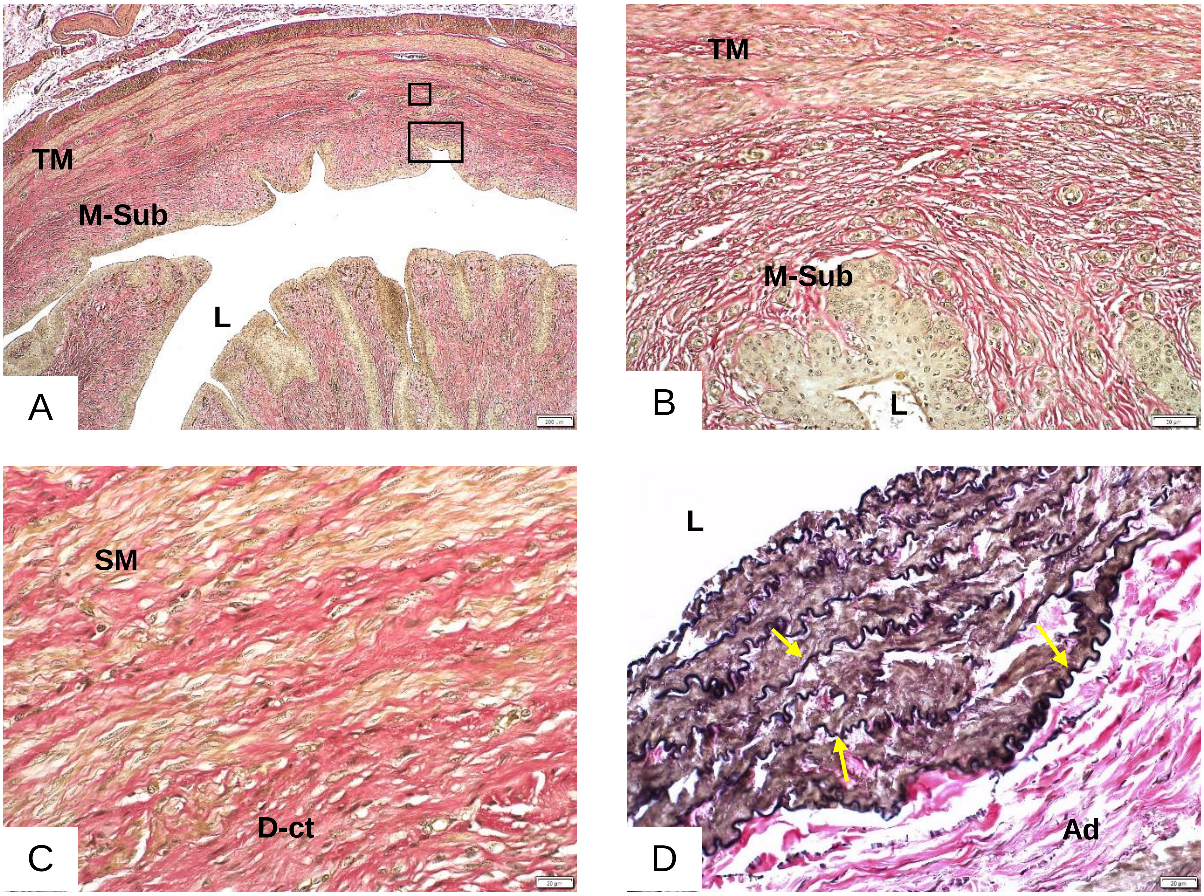


Figure 8.86: Transverse sections through the cervix highlighting the absence of elastic tissue. Verhoeffs elastic stain.

A: Transverse section through the cervix. The area outlined by the black rectangle is enlarged in B and the black square is enlarged in C. *Tunica muscularis* (TM); Mucosa-submucosa (M-Sub). Bar = 200 µm.

B: Transverse section through the cervix mucosa-submucosa. No elastic fibres are present. Lumen (L); *Tunica muscularis* (TM); Mucosa-submucosa (M-Sub). Bar = 50.

C: Transverse section through the *Tunica muscularis* of the cervix. No elastic fibres are present. Lumen (L); *Tunica muscularis* (TM); Mucosa-submucosa (M-Sub); Smooth muscle (SM); Dense, irregular connective tissue (D-ct). Bar = 20 µm.

D: Lung arteriole wall of a dog showing characteristic elastic fibres (arrows) stained black by Verhoeffs elastic stain. By comparison no elastic fibres are present in the cervix of the cheetah B & C respectively. Lumen (L); Adventitia (Ad). Bar = 20 µm.

8.7. Vagina

The vagina was a muscular tube containing longitudinal mucosa-submucosal folds that extended its entire length. The following layers were present: Mucosa-submucosa, *Tunica muscularis* and *Tunica adventitia*.

The vaginal lumen was lined by a non-keratinised, stratified squamous epithelium whose surface cells incompletely flattened in places. Irregular dense connective tissue supported the luminal epithelium and constituted the propria-submucosa which was richly supplied with small blood vessels (Figure 8.87) and contained nerves and lymphatics. Four or more large primary and some small secondary vaginal folds were apparent and were formed by folding of the mucosa-submucosa (Figure 8.88). No glands nor mucigenous cells were present.

The *Tunica muscularis* consisted of three smooth muscle layers. A thin inner longitudinal smooth muscle layer separated the thick circular smooth muscle layer from the mucosa-submucosa. The inner longitudinal smooth muscle layer could not always be clearly distinguished from the circular smooth muscle layer in places. The circular smooth muscle layer was separated from a thin outer longitudinal smooth muscle layer by a prominent vascular layer, the *Tunica vasculare* (Figure 8.89). Connective tissue separated the smooth muscle into bundles in all three layers.

The outer longitudinal smooth muscle layer was covered by the *Tunica adventitia,* an irregular loose connective tissue.

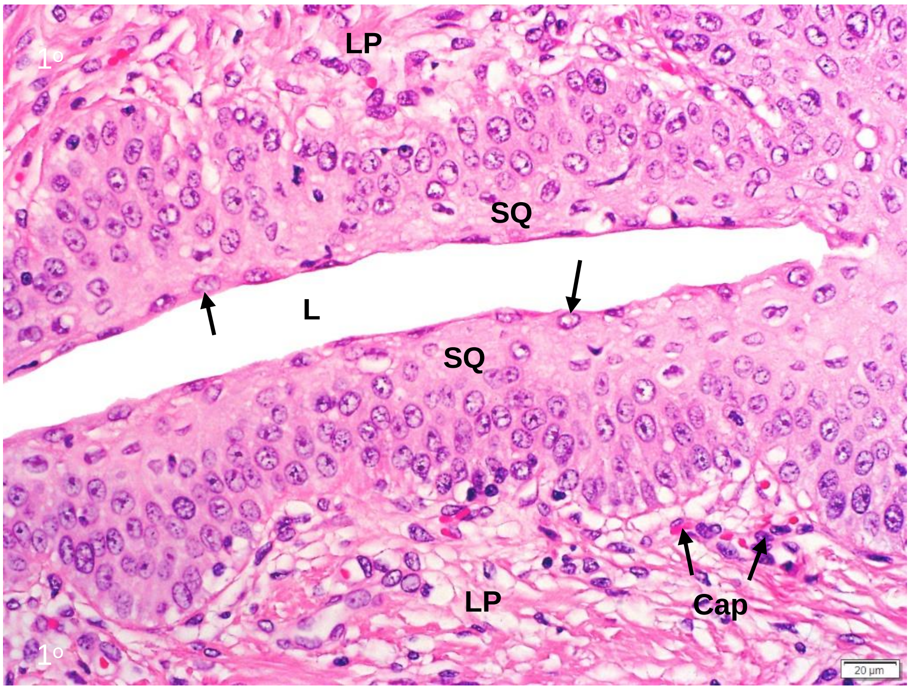


Figure 8.87: Cross section of the vagina showing the surfaces of two adjacent primary folds (1º). A rich capillary supply (Cap) is present in the *Lamina Propria* (LP). The non-keratinized, stratified squamous epithelium (SQ) incompletely flattens in places (arrows). Lumen (L); Bar = 20 µm.

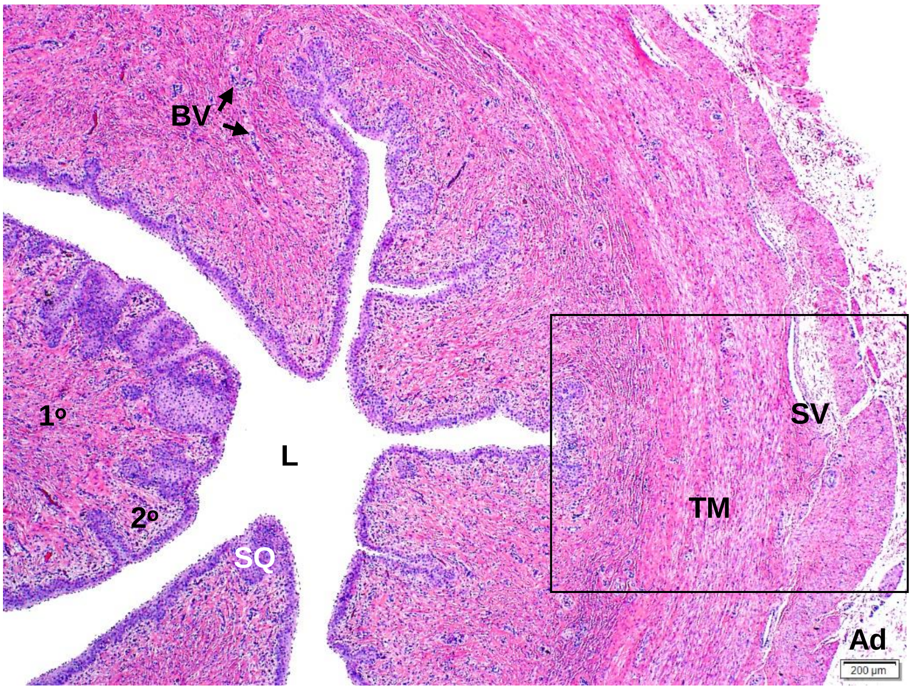


Figure 8.88: Transverse section through the cranial vagina showing prominent primary mucosa-submucosal folds (1º) with some secondary folds (2º) and a prominent *Stratum vasculare*. Numerous blood vessels supply the primary folds (BV); Lumen (L); Non-keratinized stratified squamous epithelium (SQ); *Tunica muscularis* (TM); *Stratum vasculare* (SV); Adventitia (Ad); The area outlined by the black rectangle is enlarged in Figure 8.89. Bar = 200 µm.

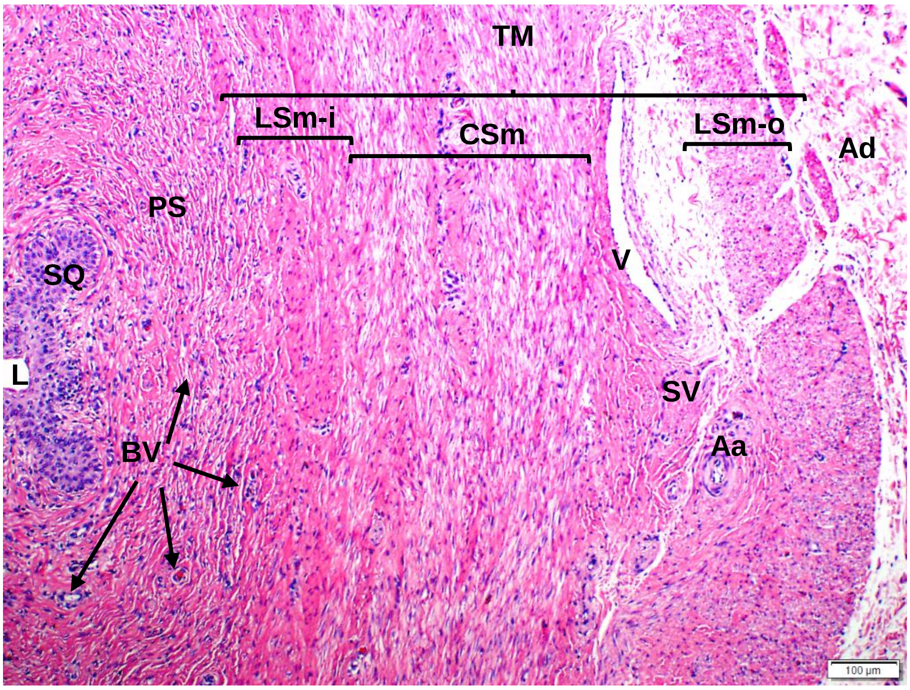


Figure 8.89: Layers of the vagina. Numerous blood vessels (BV) supply the *Propria submucosa* (PS) and a prominent *Stratum vasculare* (SV) is present. The thicker circular smooth muscle layer (CSm) is sandwiched between the thinner inner longitudinal smooth muscle layer (LSm-i) and a thin outer longitudinal muscle layer (LSm-o) respectively. Vaginal lumen (L); Non-keratinized stratified squamous epithelium (SQ); *Tunica muscularis* (TM); Adventitia (Ad); Veins (V); Arteries (Aa). Bar = 100 µm.

9. Discussion

9.1. General anatomical features of the female reproductive tract of the cheetah:

The female reproductive tract of the lioness consists of bilateral ovaries attached to the suspensory ligaments and covered laterally by the ovarian bursa. The uterine tubes run in the mesosalpinx to enter the apex of their respective uterine horns. The uterine horns are long and cylindrical, attach cranially to the proper ligament, extend caudally to unite and form the uterine body, which attaches to the cervix. The cervix attaches to the tubular vagina, which terminates at the vestibule [69]. This basic relationship of the reproductive components to each other is mirrored in the domestic cat [27] and the female cheetah.

9.2. Ligaments of the female reproductive tract of the cheetah:

In the dog, the uterus, uterine tubes and ovaries are attached to the lateral walls of the pelvic canal and dorsolateral walls of the abdominal cavity by the left and right broad ligaments. The broad ligament is broadest at the ovaries and tapers to its cranial and caudal extremities. It is further subdivided into the mesovarium, mesosalpinx and mesometrium based on the reproductive structures it contains, namely the ovary, uterine tube and uterus respectively [70]. The suspensory ligament runs in the free border of the mesovarium and is continuous with the proper ligament at the uterine extremity of the ovary. The proper ligament attaches to the tip of the uterine horn where it is continuous with the round ligament of the uterus, which continues caudo-laterally in a lateral outpouching of the mesometrium through the inguinal canal to end near the vulva [70]. The suspensory and proper ligaments are composed of connective tissue with smooth muscle fibres. The broad ligament, composed of a double layer of peritoneum, is devoid of smooth muscle and plays no role in suspending the female reproductive tract of the dog, it simply unites its components [70]. Suspension of the abdominal portion is therefore achieved by the suspensory, proper and round ligaments. The remaining portions of the uterine horns and uterine body are suspended from their caudal retroperitoneal attachments in the pelvic canal and not supported by any extrinsic muscles or ligaments [70].

In the cheetah, the broad ligament has been observed to similarly attach the ovaries, uterine tube and uterus to the dorsolateral wall of the abdominal cavity and lateral walls of the pelvic canal. The suspensory ligament extends from the dorsolateral body wall (personal communication Dr L. Du Plessis) in the free border of the mesovarium to join the proper ligament at the uterine extremity of the ovary. The proper ligament attaches to the tip of the uterine horn where it is merges with the uterine sling. The round ligament extends in a lateral outpouching of the broad ligament from near the tip of the uterine horn to the inguinal canal (personal communication Dr L. Du Plessis). Suspension of the abdominal portion of the female reproductive tract of the cheetah is therefore achieved by the suspensory, proper and round ligaments. The remaining portions of the uterine horns and uterine body are suspended from their caudal retroperitoneal attachments in the pelvic canal. However, in contrast to the dog, these latter structures are additionally supported by the uterine sling, a smooth muscle band supporting the uterus. The uterine sling is one of the most striking features of the splanchnology of the reproductive tract of the female cheetah and extended, in the mesometrium flanking the uterus, from the attachment of the proper ligament at the tip of the uterine horn to the cervix. Histologically the uterine sling was almost entirely composed of smooth muscle. The round ligament of the cheetah attached near the tip of the uterine horn where the former abutted the uterine sling but histologically did not attach to it. However, it could not be established histologically or macroscopically whether the proper ligament and round ligament were continuous with each other proximal to the attachment of the round ligament to the uterine horn, as is the case in the dog [70]. This was due to the presence of the uterine sling in the cheetah which macroscopically merged with the aforementioned ligaments. Unlike the lioness [26] no striated muscle was observed in the round ligament of the cheetah. However, since it is unknown how close to the body wall the round ligaments were incised at post-mortem, the potential presence of striated muscle in the round ligament in the vicinity of the inguinal ring of the cheetah remains to be investigated.

The broad ligament of the cheetah differed from that of the dog in that the former was composed almost exclusively of a microscopic sheet of smooth muscle that was continuous with the uterine sling, round ligament and myometrium while still containing the constituents mentioned in the dog [70]. The continuation of the myometrium, vasculature and perimetrium into the mesometrium in cheetahs is described in domestic dogs and cats [71]. Any structure containing smooth muscle fibres has the ability to lengthen and shorten to varying degrees. This movement has the potential to change the shape and / or position of the structure. It is therefore likely that the broad ligament in the cheetah plays more than a ‘uniting’ role of the reproductive tract components, the high smooth muscle content suggesting it adopts an active role in suspending and stabilising the uterus, uterine tubes and ovaries. All the ligaments (suspensory, proper, round, intercornual and broad ligament) of the reproductive tract of the female cheetah were composed almost exclusively of smooth muscle which was consistent with the situation in the domestic cat, except that the composition of the intercornual ligament is not mentioned in the cat [27]. Based on the smooth muscle composition these ligaments would be better classified as muscles. This has been proposed in the human literature for the round ligament [62]. The abdominal portion of the reproductive tract of the female cheetah was therefore suspended and supported along its entire length by a substantial continuous smooth muscle structure comprised of the broad, suspensory, proper and round ligaments, and uterine sling. Rather than passively suspending the intra-abdominal reproductive tract of the female cheetah the aforementioned structures should, due to their substantial smooth muscle components, be able to actively, albeit unconsciously, manipulate the position and or shape of the components of the reproductive tract. The degree of manipulation would be contingent on the weight of the suspended reproductive component relative to the strength of the smooth muscle acting on it.

The uterine sling has been described in the lioness where it was hypothesised that its function may be to retract and stabilise the gravid uterus closer to the lumbar spine during hunting. This might provide some protection to any foetuses by the lumbar spine and musculature providing a physical barrier to attack by horns, and allowing the female to still effectively hunt during late pregnancy [26]. However, physical elevation of the uterus close to the lumbar spine in cheetahs would potentially jeopardise the foetuses by subjecting them to the impact of a rapidly flexing and extending bony structure during hunting. A more plausible explanation may be that the uterus remains nestled in the soft abdominal viscera and the uterine sling simply increases the inertia of the uterus thereby helping to stabilise it during running (Personal communication Prof. M. Crole). Both theories hold merit. The investigator is of the opinion that the main function of the uterine sling is to assist in stabilising the pregnant uterus during hunting. This is achieved by the following mechanisms.

Firstly, the uterine sling is continuous with the proper ligament and suspensory ligament which together form one functional unit that represents a hammock. The main suspension points of the hammock, from a load bearing point of view, being the origin of the suspensory ligament, presumably on the middle or ventral thirds of the last one or two ribs[41], and the retroperitoneal reproductive tract located in the pelvic canal. The round ligaments, attaching to the proximal third of the uterine horns and extending through the inguinal canals, function as guy ropes which act to decrease the side to side sway of the uterine horns. The broad ligaments form the sides the hammock and stabilise the reproductive tract along its length by effectively forming continuous guy ropes that abut one another. The reproductive tract (ovaries, uterine tubes and uterus) is suspended in this smooth muscle hammock, referred to as the uterine hammock from here on and composed of the suspensory, proper, broad, round ligaments and the uterine sling.

The less pendulously a hammock is hung the less apt it is to swing from side to side. This is as a result of the material of the hammock being under tension on its long axis and thus capable of resisting swaying, as well as the hammock being positioned closer to its rotational-axis (the line drawn between the 2 anchor points of the hammock) which requires a larger force to displace its ventral midpoint laterally for any given distance compared to a more distantly positioned midpoint (more loosely hung or pendulous hammock). The more guy ropes a hammock has the more stable it is and the better it is able to resist swaying, as any sideways force exerted on the hammock must overcome the counteracting forces of the guy ropes to induce swaying. Therefore, it can be stated that the force required to overcome the inertia of a hammock and induce swaying motion in any given direction is directly proportional to the tension the hammock is under.

Secondly, the uterine sling is effectively a substantial muscle that is approximately the width of the uterus in the non-pregnant cheetah. Contraction of the uterine sling would do one of two things, depending on the weight of the uterus and strength of the muscle.

Scenario one: Contraction of the uterine sling is powerful enough to overcome the counteracting weight of the uterus, and shortening of the uterine hammock occurs moving the uterus closer to its rotational axis. This is most likely to occur in the non-pregnant or early pregnant uterus.

Scenario two: Contraction of the uterine sling is not powerful enough to overcome the counteracting weight of the uterus and shortening of the hammock does not occur, and the uterus does not change position. This is likely to occur during late pregnancy when the weight of the foetuses and associated fetal fluids would probably exceed the capabilities of even a hypertrophied uterine sling. In this scenario, even though the uterus does not change position, the uterine sling helps stabilise the uterus by increasing uterine inertia by placing longitudinal tension in the uterine hammock. Any force applied to the uterus must now overcome both the weight of the uterus, the friction of the uterus against the abdominal floor and the tension provided by the uterine sling.

The anatomical position of the rotational axis of the uterine hammock is important. When a cheetah is sprinting the lumbar spine is rapidly flexing and extending. Any structure ventral to, but within the range of motion of the lumbar spine, would have to oscillate in harmony with the lumbar spine to prevent injury. Any structure in motion has an area inside it, that for that particular movement, is relatively inert. This zero point (centre of gravity) is the point around which all other points move relative to it. This also applies to the sprinting cheetah. There will be an area inside the abdomen where movement is at a minimum relative to the other points. This investigator suspects that the rotational axis of the uterine hammock closely correlates with this area and that the early pregnant uterus is thus held close to or within this region. This would afford the greatest inertia and protection from centrifugal forces to the early developing foetuses in the period when they are most vulnerable. As the foetuses develop and increase in weight the uterine hammock would stretch downwards, the smooth muscle acting like a bungee cord and very effective shock absorber protecting the foetuses from excessive forces. With increased size and weight the uterus would further descend towards the ventral abdominal wall, displacing the abdominal organs cranially and laterally. The mesometrial margin of the uterus would still be located relatively close to the rotational axis as the uterus has effectively grown ventrally from this position. The abdominal viscera now surrounding the uterus would further aid in providing a soft, mobile structure that could further stabilise the uterus and cushion it from impacts. During late pregnancy the uterine horns would be the most pendulous structures. The uterine sling would aid in moving or maintaining the uterine horns closer to the rotational axis and midline, aiding stability and minimising motion within the abdomen. This would greatly aid the female cheetah during hunting as an eccentrically placed or pendulous uterine horn could throw her off balance. By centralising and stabilising the weight of the uterus closer to the centre of gravity the female cheetah minimises the deleterious effects of a late pregnant uterus on agility.

Since the cheetah is primarily a high-speed chase and capture predator [35], as opposed to an ambush predator, this functionality might be crucial to successful hunting. Female cheetahs are solitary [72], hunting and raising their cubs alone. It is therefore important for them to be able to hunt effectively late into their pregnancies and soon after birth to sustain lactation. The ability to stabilise the gravid or involuting uterus would be distinctly advantageous as cheetahs hunt smaller and more agile prey than lions normally do [72,73]. The utrine sling might also aid in emptying any remaining blood and fluid immediately postpartum by elevating the distended empty uterus off the abdominal floor, possibly decreasing the risk of associated uterine pathologies.

In humans, hypertrophy of the round ligament during pregnancy has been observed and it is recognised to play an important stabilising function on the gravid uterus [62]. Although cheetahs are not bipedal, the fact that the human uterus needs to be stabilised in a species that does not need to be mobile to survive during late pregnancy, highlights the importance of stabilising the uterus in a highly mobile species like the cheetah. Hypertrophy of the smooth muscle ligaments and uterine sling in the cheetah would be the most likely mechanism of achieving this effect during pregnancy but remains to be investigated.

The branching of the suspensory ligament observed in the cheetah has not been described in other carnivores [27,69]. The tremendous variation associated with this ligament both within and between individuals was surprising given the apparent genetic paucity in cheetahs [11]. The mechanism of development of this variation is speculative. Further research would need to be done to determine if genetics or other local factors determine the presence or extent of the development of the side branch. Since the suspensory ligament side branch was primarily composed of smooth muscle and was an integral component of the mesosalpinx, its contraction likely causes movement of the mesosalpinx acting to pull the ovarian bursa medially and dorsally against the dorsolateral surface of the ovary. This would aid in keeping the ovarian bursa with its associated fimbriae in intimate contact with the ovary facilitating ovum pickup. Another effect of contraction of the suspensory ligament side branch may be inferred from its insertion, abutting the isthmus of the uterine tube where the isthmus entered the tip of the uterine horn. Contraction may exert an effect on the isthmus possibly facilitating sperm transportation. In bovines a smooth muscle ligament, the infundibulo-cornual ligament, is present stretching between the infundibulum and terminal isthmus [74]. When contracted, the suspensory ligament of the bovine regulates the degree of flexure of the uterotubal junction [68] or plays a role in the process of ovum pickup [75]. The side branch of the suspensory ligament may play a similar role in the cheetah.

The cranial and caudal attachment points of the mesosalpinx to the suspensory ligament and tip of the uterine horn or proper ligament showed marked variation between and within individual cheetahs. This variation resulted in a minority of ovarian bursal openings (14.3%) being small and the fimbriae in close contact with the ovarian surface. This variation has not been noted before in the cheetah. The majority of ovarian bursal openings were very long, leaving the free margin of the mesosalpinx free to move away from the ovary. How this might affect the ability of the fimbriae to trap ova is unknown, but is seems possible that some ova could be lost to the abdominal cavity. In the dog the turgid fimbriae block the bursal opening preventing ova from escaping into the abdominal cavity [41]. However, the domestic cat has a more extensive bursal opening than the dog and the mesosalpinx routinely covers only the lateral surface of the ovary [76] similar to that in the cheetah.

The presence of smooth muscle bundles in the fimbriae and throughout the mesosalpinx of the cheetah should allow the ovarian bursa to contract and keep the fimbriae in close contact with the ovary. The smooth muscle components associated with the mesosalpinx attachments to the uterine tube have not been described in other carnivores. The fimbriae are presumed to be motile due to the smooth muscle component, and the rich capillary network present suggests that they may also become engorged during ovulation as is the case in the domestic dog and cat [71,77]. The lack of fat accumulation in the mesosalpinx is similar to that described in the domestic cat [76]. The literature is silent on the reasons for the lack of fat in the ovarian bursa of the domestic cat. The investigator suggests that an accumulation of fat in the mesosalpinx of the cheetah would interfere with the movement of the mesosalpinx and, to a lesser extent, the fimbriae, by the following mechanisms. The accumulated fat would change the flexibility of the ovarian bursa with a thicker walled bursa being more turgid and less able to conform to the surface of the ovary. Additionally, the increased weight of the bursa would potentially hamper the ability of the smooth muscle in the mesosalpinx to manoeuvre the bursa over the ventral and medial surfaces of the ovary, limiting contact of the fimbriae to the lateral surface of the ovary thereby increasing the risks of not trapping ova.

9.3. The Ovary:

The histology of the ovary and the process of folliculogenesis closely resembled that of the domestic dog and cat [44]. The germinal epithelium was similar to that of the domestic cat and originated as a simple cuboidal epithelium[78]. In contrast to some other species it did not become a simple squamous epithelium with age [44]. In this investigation all cheetahs investigated to age 10.5 years had a simple cuboidal epithelium lining the ovaries. The *Tunica albuginea* was similar to other domestic animals and was oriented in a lamellar fashion in places[44]. The *Tunica albuginea* of the cheetah closely resembled that of the lioness [26].

Primordial follicles were present in all cheetahs as in the domestic cat [46]. The impression gained through subjective observation was that the older cheetahs (six of seven cheetahs, aged 7-10.5 years) had noticeably fewer primordial follicles than the youngest cheetah (one of seven cheeatahs, age 3 years) and this was consistent with other research which showed a decrease in the ovarian follicle functional reserve with age [18].

There were relatively few primary and secondary follicles in these cheetahs similar to what was observed in the lioness [26]. Tertiary follicles were present in some cheetahs. This is not surprising, as primordial follicles represent the majority of follicles in the domestic cat ovary and only a cohort of primordial follicles are recruited to undergo further development during the reproductive cycle [23]. No signs of previous ovulations were present (*Corpora albicantia* nor *haemorrhagica*) in these cheetahs. As the cheetah is primarily an induced ovulator that sometimes exhibit spontaneous ovulation [19,20] this likely explains why no Graafian, pre-ovulatory follicles nor *Corpora albicantia* were documented, as no male cheetahs were housed with the female cheetahs examined and therefore would not have been recently mated.

Multi-oocyte follicles are reported as a common finding in new-born and young domestic cats [23], and are sporadically observed in the ovaries of most mammalian species [10]. They are thought to form as a result of aberrant or incomplete germ cell cyst breakdown during the earliest stages of follicle formation. Although multi-oocyte formation might also occur if the rate of growth of the oocyte exceeds the rate of somatic cell differentiation, resulting in insufficient numbers of somatic cells to surround each oocyte [23]. No multi-oocyte follicles were observed in the cheetahs studied. This is likely a function of older age (six of seven cheetahs, age 7-10.5 years), with associated decreased numbers of ova ovulating [18] and a limited sample size (7 cheetah) examined minimising the chances of detecting multi-oocyte follicles. New-born and young cheetahs would need to be examined to exclude the occurrence of multi-oocyte follicles in the cheetah.

No follicle classification system has been proposed for the domestic cat [58] or for any wild felids. Bristol-Gould and Woodruff have adapted criteria used in humans and mice for the classification of follicles in the cat [58]. Their classification system is based on the work of Lintern-Moore et al [56] work and focuses primarily on the morphology of the cells. This reflects functional development of the follicle better than systems relying on follicle or oocyte size or follicle cell numbers alone as these could vary between species. Histology textbooks and articles use various terms to divide the continuum of follicle development into easily recognisable stages. Terms are often used interchangeably and synonyms can be confusing as they do not always refer to the same morphological start and end point. This lack of clear definition and standardisation of terms makes comparison of data between authors challenging as the reader needs to become familiar with the authors definition of each term or use of an unknown term. The use of the proposed feline follicle classification system for the cheetah was successful. All categories of follicles, except for those only present post coitus (Graafian & Pre-ovulatory follicles), were easily identified and classified without ambiguity. Adoption of this new feline follicle classification system would facilitate comparison of data between authors.

The striking similarities between folliculogenesis in the domestic cat and the cheetah suggest that similar underlying hormonal control mechanisms exist to regulate the process. As new discoveries are made regarding folliculogenesis control mechanisms in the domestic cat this information can be extrapolated to the cheetah. This should prove useful in future attempts to develop ART for cheetah breeding programmes.

In the domestic dog and cat interstitial endocrine cells [49] are derived from the *Theca interna* of atretic antral follicles or from granulosa cells of atretic preantral follicles and are located in the cortex [46]. They are thought to supply prepuberal animals with oestrogens necessary for the development of secondary sex characteristics. They may also be a source of oestrogen associated with the postovulatory period [44,45]. Interstitial endocrine cells were abundant in the cortex of the cheetah and were often observed mingled with the hypertrophied, epithelioid like cells of the *Theca interna* of atretic follicles. Interstitial endocrine cells were not found in the ovaries of pre-ovulatory lionesses (3 years of age) despite the presence of tertiary follicles and many atretic follicles [26], which might indicate that a follicle needs to ovulate before they can form, although further research in the lioness, especially the documentation of interstitial endocrine cells, needs to be done to support or refute this idea.

The *Rete ovarii* have been described as being located in the medulla of carnivores and ruminants [47]. They are most often found within the hilus but may also be found within the mesovarium [49]. *Rete ovarii* are derived from the mesonephric tubules, and consist of channels lined by cuboidal epithelium or short, solid cords of epithelial cells [44,46–48]. In the cheetah the *Rete ovarii* were located in the hilus area but consistently infiltrated the suspensory ligament near the tubal extremity of the ovary. They extended cranial to the ovary inside the suspensory ligament in some cheetahs. Their function remains speculative although it has been suggested they might differentiate into follicular cells if juxta positioned to oocytes[79].

Atresia is the process by which less developed follicles that do not ovulate during any given cycle, and multi-oocyte follicles, undergo atresia. In the domestic dog and cat, follicles at any stage of the development cycle can undergo atresia. Follicles in an advanced stage of development that undergo atresia leave a scar called the *Corpus atreticum*. Primordial follicles do not leave a scar and simply undergo dissolution [44,45].

In the cheetah, similar to humans [55], all stages of atresia were present and readily encountered in most ovaries. Follicular atresia is poorly described in domestic mammals [44,45] compared to humans[55] and the lioness [26] . A comparison between women and the lioness was therefore made and tabulated to facilitate direct comparison of atretic stages (Table 9.1).

Table 9.1: A comparison of follicular atresia between women [55], cheetah and the lioness [26].

Human	Cheetah	Lioness
Very early atresia • *Theca interna* and *Membrana granulosa* are intact but some granulosa cells start sloughing into the antrum. • Follicular fluid is still present. • Degeneration of the ovum is advanced. • A remnant ovum with a swollen *Zona pellucida* is seen free in the antrum.	***Very early atresia*** As for humans except that: • The granulosa cells do not slough.	***Very early atresia*** As for humans.
Early atresia • *Theca interna* is still present but its cells have started to hypertrophy. • *Membrana granulosa* is absent having sloughed and been resorbed. • Follicular fluid is still present. • The hypertrophied glassy membrane is present and represents the thickened, folded basement membrane between the granulosa and *Theca interna* layers. • Loose connective tissue is growing in from the stroma and partially filling the reduced antrum.	***Early atresia*** As for humans except that: • The *Membrana granulosa* is present and the granulosa cells closest to the antrum hypertrophied and became pyknotic, presumably playing a role in resorbing the follicular fluid, and resulting in a collapse of the antral cavity. • The basement membrane had not started to hypertrophy yet.	***Early atresia*** As for humans except that: • The thickening of the glassy membrane is far less outspoken. • Distinct layering of the *Theca interna* cells is present.

Advanced early atresia - This distinction is not made in humans and the atretic processes described under early atresia spans this phase.	***Advanced early atresia*** - The cells of the *membrana granulosa* continued to resorb follicular fluid until the antrum was completely filled by them (*Figure 8.40*). - Pyknosis of the granulosa cells advances. (*Figure 8.40*; *Figure 8.41*). - The basement membrane starts thickening in larger follicles (Class D_1 and bigger) to form the glassy membrane and reached its most hypertrophied state (*Figure 8.41*; *Figure 8.42*). No glassy membrane formed in smaller atretic follicles. - Loose connective tissue and capillaries started to grow in from the stroma and *Theca externa* to start infiltrating and replacing the granulosa cells that filled the antral cavity similar to the early atretic phase in humans. - The glassy membrane folded together with hypertrophy and was resorbed in places (Figure 8.42; Figure 8.43; Figure 8.45). Fibroblasts were seen crossing the glassy membrane together with capillaries (Figure 8.44).	***Advanced early atresia*** Although this distinction is made in the lioness the atretic processes described better fit under early atresia for purposes of this comparison and, similar to women, the continuum of atretic changes spans this phase.
Moderate atresia - Stroma starts replacing the *Theca interna*. - The glassy membrane becomes thicker and more folded. - Connective tissue with small blood vessels completely fills the former antrum.	***Moderate atresia*** As for humans except that: - The glassy membrane regresses and disappears completely in most cheetahs. Remnants of hypertrophied glassy membranes remain for some time as the only indication of former atresia and are eventually completely resorbed leaving no scar tissue (*Corpus atreticum*). These remnants occurred mainly deeper in the cortex suggesting they may only form occasionally from large tertiary or Graafian follicles.	***Moderate atresia*** As for humans except that: - The glassy membrane regresses and disappears completely.

Late atresia	***Late atresia***	***Late atresia***
• The connective tissue is replaced by stroma. • The glassy membrane remains for some time as the only indication of the former follicle.	As for humans except that: • The glassy membrane is absent having been completely resorbed during moderate atresia. Atypical cases • Occasionally, the glassy membrane is not invaded by fibroblasts nor resorbed in places. Instead, it shows marked hypertrophy and folding. Only one example of this was found in the 7 cheetahs and 70 ovarian slides examined (Figure 8.44).	Not described in the lioness.

Atresia in the cheetah has similarities with women and the lioness. There are also some differences. Notably the *Membrana granulosa* cells remain present for most of the atretic process in the cheetah. The glassy membrane may only hypertrophy in places and not to the extent noted in women. Folding of the glassy membrane is also not as extensive as in women, except in exceptional cases. Additionally, the glassy membrane does not appear to form in early follicle classes although it can be argued that this was simply not observed in this study. The ingrowth of connective tissue is not as extensive as in women and the lioness and occurs later in the atretic process in the cheetah.

A larger population of cheetahs would need to be sampled to confirm which of the atretic process variations observed represent the general population, especially with regard to the formation and resorption of the glassy membrane. This will help answer the question as to whether the extensive folding and hypertrophy of the glassy membrane observed in cheetah 5, aged 10 years; Figure 8.51 and the classification of that novel occurrence as late atresia is truly an anomaly. The investigator is of the opinion that the splitting of early atretic changes into early and advanced early atretic changes is useful as a comparative tool in highlighting differences as to when the glassy membrane develops in women, the cheetah and the lioness.

The presence of POCs is considered an incidental finding in the cheetah and they are not considered to be of any reproductive importance [80,81]. They originate from mesonephric duct or paramesonephric duct remnants [81]. The high incidence of POCs (six out of seven cheetahs; 85.7%) confirms the 76.2% incidence reported from a similarly aged cheetah population sourced from the same Namibian establishment [81]. The POCs were primarily located at the cranial flexure of the ampulla which corroborates the findings previously reported from this Namibian facility [81].

9.4. The Uterine tube:

The uterine tube generally resembled that of the domestic dog and cat, consisting of an infundibulum, ampulla and isthmus with a uterine part [71]. The *Tunica muscularis* varied from the domestic dog and cat [71] in that it had a prominent, well developed outer longitudinal smooth muscle layer associated with the infundibulum, ampulla and isthmus which continued into the mesosalpinx. This degree of development of the longitudinal smooth muscle layer has not been described in other mammals [71].The cheetah's *Tunica muscularis* additionally varied from that of the domestic dog in that it remained a constant thickness throughout the infundibulum and ampulla of the uterine tube only increasing in thickness in the isthmus. It is thin in the domestic dog proximally and progressively thickens distally [77]. In the domestic dog the *Tunica muscularis* reaches its greatest development in the isthmus [77]. In these cheetahs the *Tunica muscularis* was well developed along the entire uterine tube. However, similar to the domestic dog, the circular smooth muscle layer in the cheetah was markedly thicker in the isthmus, specifically the uterine part of the isthmus, compared to the other regions of the uterine tube.

Macroscopically, the isthmus of the cheetah resembled that of the lioness which possesses an isthmus that opens into the apex of the uterine horn [69]. The cheetah is similar to the dog, rat, pig-tailed monkey and rhesus monkey in possessing an intra-mural portion (*Pars uterina*) of the isthmus [68]. The cow, sheep, rabbit and pig do not possess an intramural component [68]. In the cheetah, a conspicuous blood vessel was visible on the surface of the isthmus and ampulla and has not been mentioned in other species.

The surface epithelium of the uterine part of the isthmus in the cheetah was similar to that in the dog and rat, containing only non-ciliated cells [68]. In species such as the cow, sheep, rabbit and pig the surface epithelium consists of both ciliated and non-ciliated cells [68]. Detail of the uterine part of the isthmus is lacking in the literature for the domestic cat and lioness making comparison with the cheetah impossible.

The *Lamina Propria* of the isthmus in the cheetah resembled that of the dog, rat, rabbit, cow, sheep, pig, pig-tailed monkey and rhesus monkey and consisted predominantly of dense connective tissue between the luminal epithelium and tunica muscularis. Its outer surface was even and the inner surface formed the bulk of the uterine tube folds [68].

Similar to the pig, the dog and the cat, the primary folds of the isthmus of the cheetah broadened and flattened in the intrauterine part of the isthmus [68,82] . Diverticula associated with blind ending crypts are present in the terminal isthmus abutting the uterine ostium in cows[83] and are also reported in the rat, dog, rabbit, sheep, cow, pig, pig-tailed monkey and rhesus monkey [68] but were not observed in the cheetah. However, scanning electron microscopy could be used to definitively confirm their presence or absence.

In the human [84], bovine [83], rat, sheep and the rabbit [68] the intramural part of the isthmus is associated with an additional inner longitudinal muscle layer which was not present in the cheetah. In this regard the cheetah is similar to the pig, the dog [68] and the cat [82] which also lack a definitive inner longitudinal smooth muscle layer in the terminal isthmus although all species possess an outer longitudinal smooth muscle layer. The uterine tube of the dog and rat projects into the uterine lumen ending in a mound or papilla, respectively (see below). In the cow, sheep and monkeys the uterine horn simply tapers into the uterine tube [68].

9.5. The Utero-tubal junction:

The morphology of the UTJ of the dog, rabbit, rat, sheep, pig, cow, pig-tailed monkey, rhesus monkey [68] and horse [85] has been described. The basic morphology of the uterine tube of the domestic cat and lioness has been described, although a detailed description of the UTJ is lacking [27,69,82]. The uterine tube of the cheetah opened into the uterine horn on a microscopic papilla. To the authors knowledge, this is the first time that the presence of a papilla housing the UTJ has been described in wild felids. The endometrial mound that houses the uterine ostium in the domestic dog and the papilla described in rats [68] has similarities with the papilla demonstrated in the cheetah. The uterine tube also opens from a papilla in the horse [85]. The uterine tube ends in a papilla in the cat although morphological details regarding the papilla are lacking [27]. In the lioness the uterine ostium is not housed in a macroscopic papilla [69] although the possible presence of a microscopic papilla similar to the cheetah requires further investigation . In the dog the isthmus opens through a slit like ostium on a mound that projects into the uterine lumen. The circular smooth muscle of the isthmus blends with and projects through the circular smooth muscle of the uterus into the mound. In the rat, the papilla was predominantly composed of the endometrial *Lamina Propria* with circular smooth muscle present in its base [68]. The endometrial stroma of the uterus adjacent to the uterine ostium in the dog and the rat forms most of the core of the mound in the dog and is continuous with the *Lamina Propria* of the endometrium in the rat. Uterine glands were not present in the papilla of the rat [68]. Thus the papilla of the rat and the dog is similar to that of the cheetah, except that the papilla in the cheetah additionally contains branched tubulo-alveolar glands and a zone of epithelial cells surrounding the uterine ostium that is morphologically distinct from that of the lining of the isthmus and uterine horn.

Structural variation around the uterine ostium has been noted amongst other species. Oviductal projections are present at the ostium of the uterine tube in the pig and the rabbit. The endometrial stroma of the uterus adjacent to the uterine ostium forms a circular fold around these projections in the rabbit. In the pig, folds of the endometrium are found below the oviductal projections and contain uterine glands [68].

The branched tubulo-alveolar glands of the papilla housing the UTJ in the cheetah were morphologically distinct from the uterine glands and represent a unique finding. In the bovine, short tubulo-alveolar crypts have been described in the transition zone associated with the bottom of the diverticula in the terminal isthmus and identified as distinct to the uterine glands [83]. In equines crypts resembling glands have been noted throughout the uterine tube [85]. However, to the authors knowledge, this is the first time branched tubulo-alveolar glands have been described in the UTJ of any species. The dilations of the papillary glands, most prominent at the junction of the endometrium with the stroma of the papilla, were present in most sections through the UTJ papilla but varied in diameter. The cells lining the dilations did not show signs of pathology, however, cystic endometrial hyperplasia is a common uterine condition in older cheetahs [18]. These dilations could easily be confused with cystic endometrial hyperplasia if the uterine tip was submitted for histopathology and was sectioned through the UTJ region. Until further research can clarify the association of the glandular dilations with a pathological condition or as normal for the cheetah it would be wise for samples submitted for histopathology of the uterus to be taken at least a few millimetres caudal to the UTJ. Since this is not macroscopically visible it is advisable to sample at least 5 mm caudal to the external junction of the isthmus with the uterine horn.

The significance of the glandular and muscular nature of the papilla in the cheetah is unknown and it is presumed that the papilla plays a role with regulating the passage of sperm into the uterine tube as described in other species [86,87]. The muscular walls of the isthmus have been shown to block the movement of ova into the uterus for a number of hours post ovulation in bovines [88]. The histological finding of a thick muscular isthmus suggests a similar mechanism may exist in the cheetah. This would presumably ensure that ova are fertilised in the uterine tube in the cheetah as in the domestic cat [89]. These papillary glands may function as a reservoir for storage of glandular secretions or act as a sperm storage area prior to ovulation. This would be consistent with a report in the domestic cat where the UTJ and uterine glands act as pre-ovulatory sperm storage areas [87].

The simple cuboidal nature of the isthmus epithelium of the cheetah has been described in humans, except that a few ciliated cells are present in this region in women and the cuboidal epithelium is present only adjacent to the uterine ostium before becoming low columnar more proximally in the isthmus. In humans the cuboidal, predominantly non-ciliated epithelium transitions to the normal uterine endometrial columnar epithelium with uterine glands in the transition zone [84]. In the bovine uterine glands similarly start to appear in the transition zone[83]. In sheep there is a progressive transition between the predominantly ciliated isthmus epithelium and less ciliated uterine epithelium. The primary folds flatten and broaden to disappear in this zone. Numerous blind ending crypts are present pointing towards the ovarian end of the tube and uterine glands start to appear [90]. In the domestic cat, the epithelial lining of the uterine tube is a simple mixed columnar and ciliated columnar epithelium with a progressive decrease in columnar cell height from the infundibulum to the isthmus, with the columnar epithelium of the isthmus being half the height of the infundibular epithelium [82]. In the cheetah, the transition zone is marked by an abrupt change from a simple cuboidal or low cuboidal epithelium lining the isthmus to a simple columnar epithelium at the uterine ostium. This columnar epithelium extends approximately half way to the base of the papilla and then abruptly changes to reflect the normal simple cuboidal to low cuboidal uterine epithelium.

The stage of the oestrus cycle may influence cell morphology. In the cow both ciliated and non-ciliated cells are present in the UTJ with ciliation being increased during proestrus and oestrus[83]. In contrast, ciliation remains unchanged during the reproductive cycle in the isthmus of Thai Swamp buffalo [91] and sheep [90]. The borderline between the ciliated cells in the transition zone in the cow and the sparsely ciliated surface epithelium of the uterus shifts in a uterine direction during proestrus and oestrus and retreats during interestrous and diestrus. Additionally, the border is not clear cut and runs an intricate course with many projections emanating from either side [83]. In the cat, ciliation of the uterine tube epithelium is mediated by the presence of oestrogen and is highest during oestrus. In the presence of progesterone, during the post ovulation phase after mating or during pregnancy, many of the epithelial cells deciliate in the uterine tube and almost halve in height. Many cells also undergo apoptosis [92]. It is unknown whether cyclical changes happen in the cheetah, or whether the cells become ciliated or change height at different stages in the reproductive cycle. The stage of the reproductive cycle in the cheetahs examined is unknown but is likely to be interestrus given that the reproductive tracts displayed no evidence of pregnancy nor recent ovulations, and all the cheetah ovaries were active. It therefore cannot be ruled out that the simple low cuboidal to cuboidal epithelium of the intrauterine part of the isthmus might double in height and even develop cilia during periods of oestrogenisation. This also applies to other parts of the reproductive tract such as the uterus, cervix and vagina.

9.6. The Uterus:

The histology of the uterus of the cheetah resembled that of the domestic dog [42] and cat [42] with a few notable differences. The uterine lumen of the cheetah was lined by a simple cuboidal or low cuboidal epithelium, except for the region surrounding the apex of the papilla containing the uterine ostium which displayed a columnar epithelium. This contrasts with the domestic dog and lioness where the uterus is lined by a simple columnar epithelium [26,79] . No ciliated epithelial cells were noted in any part of the uterus of the cheetah as noted in scattered locations in the domestic dog [70]. A thin basal zone in the endometrium, as observed in domestic mammals [42], could not be appreciated in the cheetah [78]. The uterine glands in the cheetah were lined by a simple cuboidal epithelium which contrasts with that in the domestic dog, cat and lioness where the lining is a simple ciliated columnar epithelium [26,79]. However, as discussed earlier, periods of oestrogenisation in the cheetah might be associated with a change in morphology of the cells lining the reproductive tract. The non-ciliated cuboidal nature of the uterine epithelium and uterine glands might become more columnar and even ciliated in places during pro-estrous and oestrous. The variation in degree of branching of the endometrial glands in the cheetah is also likely to be associated with cyclicity, with uterine gland branching being more evident during periods of progesteronisation and straighter uterine glands predominating during anestrous or interestrous periods when sex steroid levels are significantly lower. This would be consistent with similar findings in the horse [93].

The outer longitudinal smooth muscle layer of the uterus, blood vessels and perimetrium of the cheetah were continuous with the mesometrium as described in the domestic dog and cat [71]. However, as described in the lioness [26] the outer longitudinal smooth muscle layer of the uterus was continuous with the uterine sling in the cheetah. In the cheetah, a rich nerve plexus was observed between the endometrium and myometrium which contrasts with the domestic dog and cat where nerves richly supply the perimetrium [78]. This well-developed nerve plexus abutting the endometrium in the cheetah may play an important role during pregnancy. As discussed earlier, the investigator believes the uterine sling plays an important role in stabilising the uterus and increasing the inertia of the pregnant uterus. However, too much uterine turgor might negatively impact the foetuses. Danger to the foetuses in this regard would be greatest during hunting when the cheetah is running at high speeds. Neurological feedback on the position of the uterus and the stress status of the foetuses while hunting would be important to optimise the balance of the need of the mother to effectively catch prey to provide nutritional support to the developing foetuses while not compromising the health of the foetuses. The neurological network observed may be involved with regulating acute pressure changes within the uterus through an autonomic feedback mechanism that regulates the turgor of the uterine sling.

9.7. Cervix and Vagina:

The stratified squamous epithelium of the cervix of the cheetah with some surface cells failing to flatten, lack of mucigenous and goblet cells, and very few tertiary folds is similar to that described in the lioness [26], but contrasts with the domestic dog where a simple columnar epithelium with mucigenous and goblet cells is present [78]. No secretory activity was evidenced by the absence of coagulated mucus in the lumen. This finding may be explained by a non-oestrus state since more mucus is produced during oestrus. Tertiary folds of the mucosa-submucosa described in the domestic dog [78] were not seen in the cheetah. Interestingly, the lining of the cervix in the cheetah was similar to that of the vagina. This similarity has been described in sows [78] and it remains to be investigated if the nature of the cervical lining in the cheetah also undergoes cyclical changes. Of particular interest was the total absence of elastic fibres in the walls of the cervix of the cheetah. This contrasts with the situation in domestic animals [42] and represents a novel finding in the cheetah. The lack of elastic fibres may allow the muscular cervix of the cheetah to dilate faster, by providing less resistance to expansion compared with elastic tissue, and to a greater extent during partus. This may aid in a less protracted birth process which may afford some survival benefits to the female cheetah and her offspring which are regularly predated by larger predators [12].

The histology of the vagina of the cheetah generally resembled that of the domestic dog and cat [26,79]. Similar to the domestic dog and lioness, the vagina of the cheetah was lined by a non-keratinised, stratified squamous epithelium [26,79]. As in the domestic dog and cat, but in contrast to the lioness, an inner longitudinal muscle layer of the T*unica muscularis* was visible [26,79]. Cyclical changes to the surface epithelium of the vagina of the cheetah can be anticipated as demonstrated in other domestic species [42]. The lack of a more prominent layer of superficial squamous cells reflects the low oestrogen concentrations [42] consistent with the suspected interestrous state of these cheetahs discussed earlier.

9.8. Concluding Remarks

Limitations to this study include the small sample size, skewing of age and stage of the reproductive cycle. Only 7 cheetah uteri were examined (one 3 year old cheetah uterus, one 7 year-old cheetah uterus and five 10-10.5 year-old cheetah uteri), of which the majority represented older captive cheetah. All the cheetahs showed signs of ovarian activity at the time of death, however, since none of the cheetahs had been mated, Graafian, pre-ovulatory follicles and *Corpora lutea* were not documented. The histological findings should be interpreted in light of this, namely, that these cheetahs were all in an interestrous period characterised by low progesterone and oestrogen levels. Variations in cell height and morphology may occur at different stages of the reproductive cycle as noted earlier in the discussion. Likewise, the single uterus of a 3 year-old cheetah examined, limits the strength of the outcomes for that age group. Although the histology of the 3 year-old cheetah mirrored that of its older counterparts, a greater number of younger cheetah uteri would have been useful to confirm this observation. It is currently unknown whether there are any histological differences between the female reproductive tracts of the examined cheetahs (all wild born and captive raised) versus captive born and raised, and wild born and raised female cheetahs, another limitation of this study.

An explanation for the artificial lucencies, described in various slides, especially around the uterine glands remains to be determined. Fixation of entire uterine tracts without directly exposing the uterine lumen to formalin as well as prolonged fixation in formalin may play a role in this phenomenon. In many histological sections the *Membrana granulosa* separated from the basement membrane in places. Similar appearing changes were evident in another text but not commented on by the authors [94] leading the investigator to assume that the changes were considered artefactual.

Further research needs to be done to document the histology of the vestibule and clitoris, document the histology of the uterus during oestrus and anoestrus, characterise the innervation and lymphatic supply to the female reproductive tract as well as to determine the presence or absence of striated muscle in the round ligament in the vicinity of the inguinal canal.

Despite the short comings outlined above, it was clear from the present study that the histological features of the female reproductive tract of the cheetah generally resembled that of the domestic dog and cat [24,42,82]. However, variations in the suspensory ligament and ovarian bursa, the presence of branched tubulo-alveolar papillary glands and lack of elastic fibres in the cervix of the cheetah has not previously been described in any other species to the investigators knowledge. These novel findings highlight the importance of documenting the histology of related species.

Proof of concept has been established for the use of a feline specific follicle classification system. Adoption of this system will clear up some of the confusion associated with previous systems and aid in the comparison of data between researchers.

The histology reported will aid pathologists in interpreting biopsies from specific regions of the uterine tract and aid the investigator and other researchers in future development of clinical tools to evaluate reproductive health in the female cheetah.

10. References

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11. Annexures

11.1. Annexure A

Data capture form - Gross dimensions of the formalinised cheetah ovaries in millimetres.

	Ovary Length		**Width (medial to lateral surface)**		**Height (mesovarium to free margin)**	
	Left	**Right**	**Left**	**Right**	**Left**	**Right**